# THE EFFECT OF VINEYARD MANAGEMENT PRACTICES ON PINOT NOIR FRUIT AND WINE COMPOSITION

Fiona Kerslake B.Agr.Sc. (Hons)

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# ABSTRACT

The current study was designed to investigate commercially feasible vineyard management practices across a range of intensities to evaluate the effects of these practices on Pinot Noir fruit and wine composition. Over three growing seasons, 2006, 2007 and 2008, two aspects of crop load management (node number retained at winter pruning and bunch removal) and two aspects of canopy management (leaf removal and shoot trimming) were investigated on a Scott Henry training system. To evaluate the effect on fruit and wine composition, individual treatments in the field were harvested, processed and fermented separately. Ferments were approximately 10 kg in size and winemaking was carried out at the micro winery at Tamar Ridge Estates' Kayena vineyard.

Winter pruning levels of 10, 20, 30 or 40 nodes per vine were applied to the same vines for consecutive seasons. The bunch removal trial removed 20 % or 40 % of bunches at four different stages of the season: fruit set, pea size, 10 % veraison and 90 % veraison. In the 2006 season, 20 % or 40 % of leaves were removed on upper and lower shoots at four different stages of the season (fruit set, pea size, 10 % veraison, 90 % veraison). For the 2007 and 2008 seasons, four different leaf removal treatments were applied removing the basal four leaves, the middle section four leaves, the apical four leaves or removing no leaves on vines shoot trimmed to approximately 12 nodes on upper and lower shoots. The shoot trimming trial varied the length which shoots were trimmed to on upper and lower shoots. In the 2006 season, upper shoot length only was varied (6, 12 or 22 nodes per shoot) with approximately 12 nodes per shoot on lower shoots. For the 2006 and 2007 seasons, an additional treatment was added reducing lower shoot length to 6 nodes with 12 nodes per shoot on upper shoots.

Yields varied up to three-fold as a result of pruning treatments, yet the predicted decline in fruit and wine composition as a result of increasing node number was only seen in seasons of cool weather conditions. Yield was varied 1.5-fold in the bunch removal trial and only in the season of cooler than average ripening period was bunch removal effective in improving fruit and wine composition. Leaf removal as a canopy management tool was effective for reducing *Botrytis cinerea* pressure, however little fruit and wine composition differences were seen between no leaf removal and the commercial standard of the removal of basal leaves in the

fruiting zone. The shoot trimming trial indicated that reducing shoot length from the commercial standard was detrimental to fruit and wine composition, but increasing shoot length did little to improve wine composition.

The influence of weather within seasons was found to override the effects of the viticultural management practices and the responses of fruit and wine composition could be interpreted within the context of the weather conditions of each season. During seasons of warmer than average temperatures, in particular during the ripening period, viticultural management of Pinot Noir vines had little effect on fruit and wine composition. In contrast, viticultural management in cooler than average seasons did affect fruit and wine composition and may repay the investment in terms of improved wine composition and therefore quality.

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# CHAPTER 1: INTRODUCTION

## 1.1 GRAPES AND WINE

Grapes are the world's most important fresh fruit crop with the majority (66 percent) destined for wine production (Jackson 2008). The international grape and wine industry has a very long history with evidence of wine and winemaking as early as 5000 BC in Europe (McGovern et al. 1996). Wine is often seen as a status symbol, a sign of wealth, good taste or even, as in the case of collectable wines like Penfolds "Grange", as an investment. Wine prices can range from extremes of the very cheap (AUD 1.99 for a cleanskin) to the very expensive (AUD 180 000 for a 1787 Chateau Lafite Bordeaux). Of course, within the more common commercial market with a much smaller range in values, winegrape prices by region reflect the perceived wine value made from these grapes.

In production terms, Tasmania is a small player in the overall Australian wine industry with only 0.4 percent of Australia's winegrape tonnage coming from the state in 2009 (Australian Wine and Brandy Corporation 2010). The Tasmanian industry is still relatively young, with the oldest still producing vineyard being planted in 1956, and the majority of vineyards being planted between 1994 and 2009 (Figure 1-1). The value of grapes and wine from Tasmania was 1.9 percent of the national total as the Tasmanian industry was worth AUD 49 million in 2009, which reflects the high grape prices (W.F.A. 2009). Grapes grown in Tasmania are of higher value than the rest of Australia because of the cool climate and despite national surpluses, Tasmanian winegrapes continued to command premium prices.

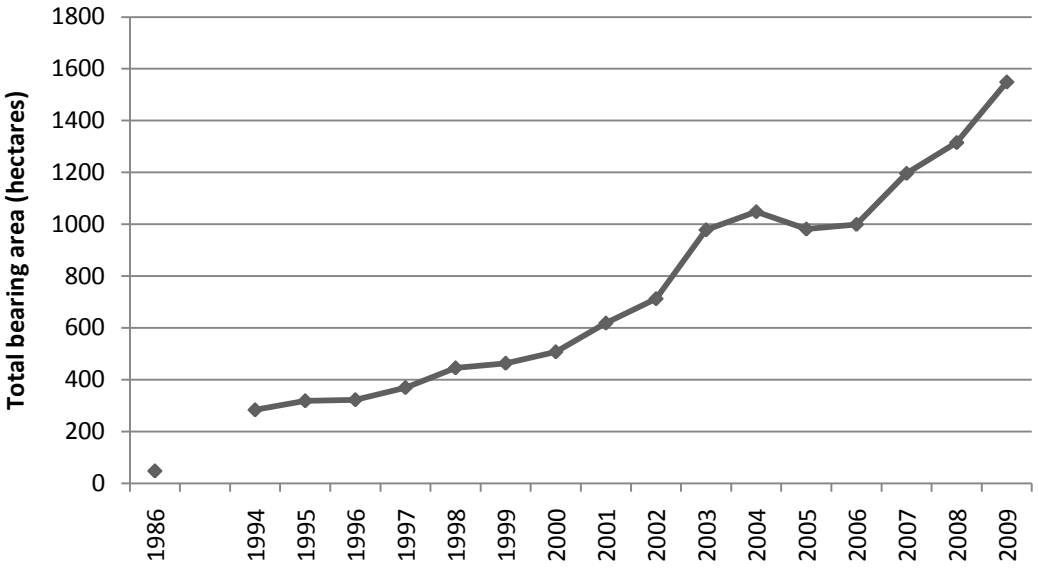


**Figure 1-1 Growth of the Tasmanian wine industry since records were commenced in 1986 by total bearing hectares (Tasmanian Department of Primary Industries Parks Water and Environment 2009).**

## 1.2 TASMANIAN SEASONAL WEATHER CONDITIONS

The Australian wine industry often refers to the classification system of climatic regions developed by Smart and Dry (1980) and the coolest category in this system is for regions with a mean January temperature (MJT) of less than 19.0 °C. This category includes the Adelaide Hills (S.A.), Macedon Ranges (Vic.), Mount Benson (S.A.), Henty (Vic.), Launceston (Tas.), St Helens (Tas.) and Hobart (Tas.) regions. For these regions MJT is across a 2.0 °C range, from 18.7 °C (Adelaide Hills) to 16.7 °C (Hobart) and other categories only span a 1.0 °C range. It is suggested therefore, that Tasmanian wine regions should therefore fall in a new 'cold climate' category of below 18.0 °C.

Howell (2001) highlights the variability between cool climate regions when specifying that regions such as Coonawarra, Napa and Bordeaux have similar season lengths to Marlborough, Hobart and Geisenheim, however the latter can be up to 400 growing season degree days (GDD) (base 10 °C) cooler. This difference in GDD can present the challenge of achieving sufficient maturity every season as a lack of heat accumulation may not always result in full sugar or desired flavour profiles.

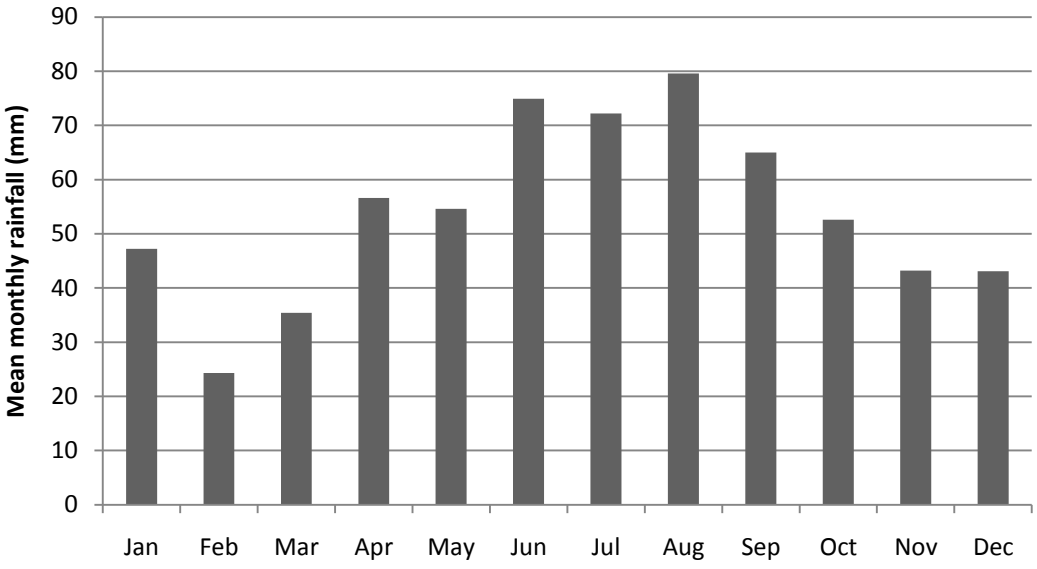


**Figure 1-2 Mean monthly rainfall for BOM Low Head weather station (Station number 091923) from 1998-2010.**

Other issues for viticulturists in cool climates, but not limited to cool climates, include cloudiness, humidity and the possibility of rain occurring around harvest time in cool climates. Tasmanian harvest usually begins in March for sparkling wine fruit and continues until May for table wines. Pinot Noir is generally picked in April or May, depending on desired maturity, and as can be seen in Figure 1-2, considerable amounts of rain generally fall in these months. This rainfall carries with it an increase in *Botrytis cinerea* bunch rot disease pressure, which is not ideal for harvest. Data in Figure 1-2 was taken from the Low Head Bureau of Meteorology weather station (www.bom.gov.au), as this is the closest station to the experimental vineyard used in the present study as explained in further detail in Chapter 3.

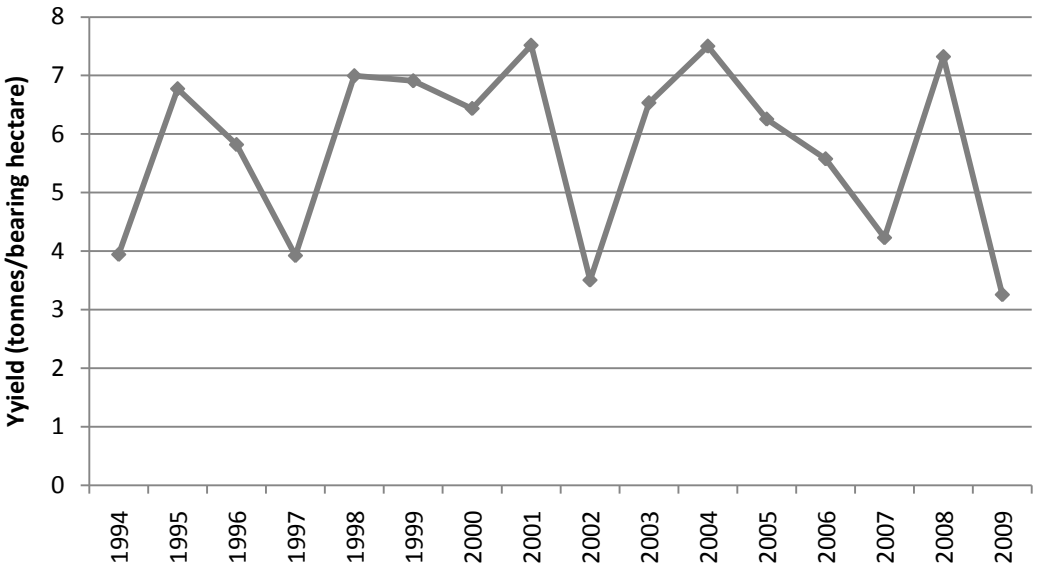


**Figure 1-3 Tasmanian average of annual yield (tonnes per bearing hectare) across all varieties from 1994-2009 (Tasmanian Department of Primary Industries Parks Water and Environment 2009).**

The cold climate of Tasmania has historically suffered large annual yield fluctuations (Figure 1-3), which was investigated by Heazlewood (2005) in her pioneering viticultural research for Tasmania. Seasonal weather conditions, such as temperature and sunshine hours at flowering and rainfall at fruit set were found to contribute to these yield fluctuations (Heazlewood 2005). Along with variation in yields, concurrent variation in wine quality has also been observed according to Langton's vintage ratings (Figure 1-4). It is generally accepted that higher yields will result in lower quality Pinot Noir wines, but there was a lack of significant correlation between Langton vintage ratings and mean annual yields per hectare for the north ($r^2 = 0.03$) or the south ($r^2 = 0.17$) of Tasmania. There have been few studies between yields in Pinot Noir and wine quality in Tasmania, and the current study has attempted to address this.

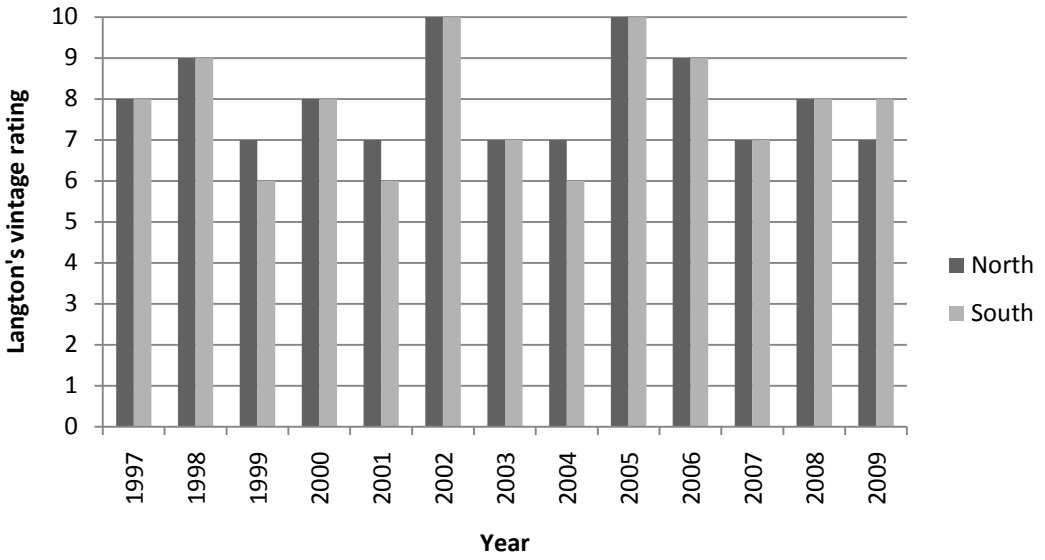


**Figure 1-4 Langton's rating of vintages across all varieties in northern and southern Tasmania (Langton's 2010).**

## 1.3 PINOT NOIR

Described as one of the hardest varieties to "get right" both in the vineyard and in the winery (Robinson 1996), Pinot Noir is one of the world's most beguiling wine grape (*Vitis vinifera*) varieties, able to both cause pain and bring reward to grape growers and winemakers alike. Some of the greatest wines in the world are made from Pinot Noir (Robinson 2006a), in particular Burgundian wines of France are celebrated. The best Pinot Noir wines of the world invariably come from cool regions, such as in Europe (for example Burgundy), North America (for example Oregon), New Zealand (for example Central Otago) and Australia (for example Tasmania) and are all capable of producing premium to super-premium quality Pinot Noir wines.

As 9 percent of Australia's total plantings are Pinot Noir, behind varieties such as Shiraz, Sauvignon Blanc and Cabernet Sauvignon (Australian Wine and Brandy Corporation 2010), it is the sixth most widely planted variety and therefore very important to the Australian wine industry. Yet, to the best of the author's knowledge, little Pinot Noir viticultural research has been carried out to date in Australian cool climates and it would seem desirable to learn how vineyard management might affect Pinot Noir wine style and composition. Jackson and

Lombard (1993) commented in their review that variety and site specific trials always need to be carried out to determine exactly how different varieties, and even different clones will perform, in different regions. The apparent lack of literature examining Southern Hemisphere Pinot Noir vineyard management practices and the lack of viticultural trials taken through to the winemaking process highlighted the need for research to be undertaken in these areas. The fluctuation in annual seasonal weather conditions had been recognised as an issue for quality Pinot Noir production (Heazlewood 2005, Howell 2001) thus highlighting the need for research to be repeated over seasons.

## 1.4 SUMMARY, MAJOR OBJECTIVES AND HYPOTHESES

Throughout the annual growth and biennial yield cycles of the grapevine, there are many opportunities for intervention with different management practices. Most of these practices involve variation in either the number of sinks, such as developing fruit, or sources, such as exposed leaves, which result in a shift in the source to sink ratio. Two common Pinot Noir yield regulation management practices of winter pruning and bunch removal and two common canopy management practices of leaf removal and shoot trimming were selected for investigation in the current study (Table 1-1). Each management practice occurs at a different time in the season and varies the source to sink ratio, thus impacting on fruit and wine composition in different ways.

**Table 1-1 Vineyard management practices investigated in the current study and the effect of increasing severity on the source to sink ratio.**

| | Sink | Source | Timing |
|---|---|---|---|
| Pruning | ↓ | ↑ | Winter |
| Bunch removal | ↓ | - | Various (generally veraison) |
| Shoot trimming | - | ↓ | Pre-veraison |
| Leaf removal | - | ↓ | Various (generally pre-veraison) |

For the present study, Chapter 1 provides a general introduction to this thesis. Chapter 2 reviews literature and provides background information as to the effects of vineyard

management decisions on fruit and wine composition across a range of cultivars, including Pinot Noir. Chapter 3 provides a description of the site used for the present study and general materials and methods common to all four experimental chapters, Chapters 4 to 7. Chapters 4 and 5 cover yield regulation management practices of winter pruning and bunch removal respectively, and chapters 6 and 7 investigate the canopy management practices of leaf removal and shoot trimming respectively. Chapter 8 considers the individual vineyard management practices in a more holistic sense and combines the results of Chapters 4-7 to better understand the relationship of these practices to fruit and wine composition.

**The main objectives of this thesis were:**

- To examine a range of winter cane pruning level treatments, varying node number per vine, on a Scott Henry training system to investigate the effect on Pinot Noir fruit and wine composition.
- To investigate a series of bunch removal treatments on a Scott Henry training system, to test if number of bunches removed or timing of bunch removal affected Pinot Noir fruit and wine composition.
- To examine a variety of leaf removal treatments on a Scott Henry training system to determine the best time and extent of leaf removal to maximise Pinot Noir fruit and wine composition.
- To investigate shoot trimming length on a Scott Henry training system to achieve an ideal canopy height to ripen a crop load for maximum Pinot Noir fruit and wine composition.

**The main hypotheses of this thesis were:**

- Does reducing the winter pruning level from a commercially regarded 'high' node number (35 nodes per vine) increase fruit and wine quality?
- Where does the balance of the source to sink ratio lie for maximum fruit and wine composition? Is it more beneficial to alter the source amount or the sink amount or, as in the case of winter pruning level, both at once?
- Is there more benefit to adjusting crop load at the beginning of the season, ie at winter pruning, rather than later in the season by removing bunches? Or is there

sufficient fruit quality improvement to justify bunch removal at different times throughout the growing cycle?

# CHAPTER 2: BACKGROUND

The relationship between yield and grape and wine quality has been studied quite extensively for varieties grown in warmer climates (Bravdo et al. 1984, Bravdo et al. 1985, Chapman et al. 2004, Clingeleffer 2009b, Howell 2001, Howell et al. 1991, Jackson & Lombard 1993, Keller et al. 2004, Keller et al. 2005, Keller et al. 2008, Kliewer & Dokoozlian 2005, McCarthy et al. 1987, Ough & Nagaoka 1984, Sipiora 2009, Smart 1982, Smart 1985, Smart et al. 1990). Fewer studies have investigated the relationship for the cool climate variety, Pinot Noir (Heazlewood et al. 2006, Petrie et al. 2000c, Petrie et al. 2000b, Petrie et al. 2000a, Reynolds et al. 1994, Reynolds et al. 1996, Zamboni et al. 1996). Further, given that there is a need to study such relationships region by region (Jackson & Lombard 1993) the question remains as to how best manage Pinot Noir vineyards in newer regions like Tasmania, Australia.

Ripening Pinot Noir in cool climate regions can be a major problem (Howell 2001) and growers employ a range of vineyard management practices to encourage adequate ripeness each season. Crop load and canopy management practices come at an additional cost each time a pass is made through the vineyard, be it by machine or person, consequently an increase in grape and wine quality needs to be achieved to justify these practices. Pinot Noir is best suited to cool climates, however the climatic variability in cold regions has a major influence on Pinot Noir yield, fruit and wine quality. In cold regions there are a variety of seasonal weather conditions that hold particular risks, like frost in spring or autumn, and in cool years crops may not adequately ripen.

## 2.1 YIELD AND YIELD COMPONENTS

While yield is the most commonly used parameter to evaluate vineyards, consideration of yield components provides a more complete understanding as to the impact of vineyard management practices. Each of the yield components defined below are able to be manipulated by management practices. The number of count nodes per vine is a measure of pruning severity, the number of shoots per count node is a measure of budburst, the number of bunches per shoot is a measure of fruitfulness and the number of berries per flower is a measure of fruitset (Tassie & Freeman 1988).

*(Yield equation from Tassie & Freeman 1988 removed)*

Berry size is considered to be of great importance to winemakers due to the belief that smaller berries make better wines (Dry et al. 1998, Matthews & Kriedemann 2006), though this is not necessarily the case. The skin to flesh ratio is greater in smaller berries and can lead to greater colour and secondary metabolite extraction during winemaking (Dunn et al. 2004). However smaller berries produced as a result of vineyard cultural practices, leading to high yield, can result in lower accumulation of secondary metabolites in the berry (Dry et al. 1998). Shiraz trials in the Barossa Valley have shown a positive relationship to wine colour and total anthocyanin content with berry size as a result of minimal pruning (McCarthy et al. 1987). Walker et al. (2005) noted that increases in wine quality due to small berry size are most likely an effect of treatments applied in the vineyard to results in small berry size, rather than 'intrinsic developmental differences between large and small berries'.

The final weight of berries and bunches is influenced not only by climatic and endogenous factors, but also directly by viticultural practices such as irrigation, pruning level and trellis type (Tassie & Freeman 1988). These cultural practices may influence the thickness of the berry skin, which determines the amount of tissue available in which anthocyanins and tannins can accumulate (Matthews & Kriedemann 2006). Matthews and Kriedemann (2006) further suggest factors influencing berry size variation are more important than berry size itself, for example smaller berries may be the result of minimal pruning or water deficit, both causing variation in fruit composition.

*Figure 2-1 (Biennial yield cycle diagram from Pearce & Coombe 2004 removed)*

The biennial yield cycle of grapevines (*Figure 2-1*) means that vineyard cultural practices in one year have an effect in subsequent years (Howell et al. 1994). The concept of vine balance is very important to maintain the equilibrium between vegetative and reproductive growth, whilst maintaining good grape quality for winemaking. This concept of balance was quantified early last century by M.L. Ravaz who used the ratio of fruit yield (Y) to the weight of one year-old wood removed at pruning (P) (Ravaz 1906). The review by Howell (2001) points out there is a fundamental problem with this approach, namely that vine balance can only be calculated

at the end of the season following pruning and does not provide any information during the season.

Further work on vine balance was carried out in the 1920's to modify the Ravaz index to become a predictive tool (Partridge 1925). Partridge (1925) used the weight of the prunings in Year 1 as an indicator of the crop load in Year 2 that could be fully ripened and he called this the 'Growth-Yield Relationship' or yield to pruning weight ratio (Y:P). This was expanded upon by a large volume of pruning level research conducted by N.J. Shaulis from the 1940's to the 1960's and is summarised by the statement 'that the larger amount of cane growth that a vine makes, the greater will be its capacity to yield fruit the following year' (Shaulis 1950).

Studies later in the 20$^{th}$ century by Bravdo et al. (1985) confirmed that yield on its own was not a true indicator of wine quality and that the ratio of fruit yield to pruning weight (Y:P) was a far better indicator. It has been reported that values ranging between 5 and 10 are ideal for 'balanced' vines (Smart & Robinson 1991), but Kliewer and Dokoozlian (2005) comment that for varieties with smaller bunches such as Pinot Noir, a range of 3-6 is optimal. The ratio is generally lower in cooler climates than warm climates (Dry et al. 2004).

In cool climates the annual yield fluctuations due to variation in weather patterns further complicate the concept of vine balance (Dunn et al. 2004, Howell 2001), with the main concern being that full maturity may not be reached in every season (Vasconcelos & Castagnoli 2000). Annual yield fluctuations in Tasmania were investigated by Heazlewood (2005) with findings that for Pinot Noir and Chardonnay temperature and sunshine hours at flowering have a greater effect on yield than the previous year's yield. Such results confirm recommendations to prune to higher node numbers than vines could normally be expected to ripen, and then to bunch thin back to target yields later in the season, such as soon after fruitset, when yield estimation is more accurate (Dry et al. 2004).

Number of nodes per vine to be retained is the first viticultural management decision in the season that directly influences yield estimation. The retention of higher than required node numbers is common because inclement weather at flowering, such as rain, cool weather or cloudiness, can greatly reduce yields due to poor fruitset (Dry et al. 2004). The potential

number of flower primordia is established in the first season and during budburst early in the second season and is driven by many factors including light, temperature, carbohydrates and nutrition (Dunn et al. 2004, Mullins et al. 1992). After fruitset, yield estimation is more accurate as it is clear how many of these flower primordia have developed into fruit as a consequence of both climate and endogenous conditions (Mullins et al. 1992, Tassie & Freeman 1988).

Budburst is primarily influenced by pruning level as pruning to low node numbers promotes the production of multiple shoots per node to a maximum of three (Smart & Robinson 1991). The failure of buds to 'burst' from count nodes will also influence yield estimation. Thinning shoots from count nodes is not a very common practice in Australia, but when utilised, is usually carried out before flowering or when shoots are around 15 – 20 cm long, mainly to remove non-fruitful shoots (Smart 1988). This operation carries the risk of low yields caused by inclement weather at flowering. Smart (1988) also notes that the shoot thinning operation in itself may cause poor fruitset, as seen in Pinot Noir and Chardonnay in cool climates, due to an increase in vigour of remaining shoots.

Canopy manipulation techniques can alter the architecture of a vine's canopy and have a large influence on the vine microclimate, which is 'the climate within and immediately surrounding a plant canopy' (Smart & Robinson 1991). An improved microclimate has been shown to improve bud fruitfulness, increase grape soluble solids, improve varietal character and colour, and to decrease vegetative flavours (Reynolds et al. 1994). Canopy architecture is also crucial in determining crop load in the following year as flower initiation and inflorescence differentiation occur in the first year of the biennial cycle and is promoted by high light intensity, so the amount of light falling on the developing buds determines the fruitfulness of that bud (Vasconcelos et al. 2009).

One of the primary effects of canopy manipulation is alteration of the leaf area to fruit weight ratio. Leaf area (LA) to fruit weight (Y) ratio (LA:Y) is well documented as being crucial to being able to fully mature a crop load in any given year. It has been suggested in cool climates such as Tasmania, a higher LA:Y ratio is required to ripen crops, as the grapes ripen more slowly than in warmer climates due to sub optimal temperatures for photosynthesis (Dry et al.

2004, Smart & Robinson 1991, Vasconcelos & Castagnoli 2000). However the leaf area to fruit weight ratio should not be used alone to determine the ripening capabilities of a vineyard as variables such as variety, climate, region and trellis type also have an influence (Dry et al. 2004).

The typical range for LA:Y is within 5 to 15 $cm^2/g$ (Dry et al. 2004). In hot climates, Kliewer and Antcliff (1970) found that 10 $cm^2/g$ was required to fully mature Sultana grapes (to 23 °Brix), whereas Tokay required 11 to 12 $cm^2/g$ to reach 20 °Brix (Kliewer & Weaver 1971). Trials by Iland et al. (1994) showed higher grape colour levels when LA:Y was increased in both Shiraz and Pinot Noir. Jackson and Lombard (1993) suggest that LA:Y is inversely related to the yield to pruning weight ratio (Y:P) as pruning weight and leaf area are well correlated (Bravdo et al. 1984, Bravdo et al. 1985, Gal et al. 1996). It is far easier to measure Y:P than LA:Y as it is non-destructive during the growing season.

Lebon et al. (2008) describe sink organs as those which attract nutrients and source organs are organs which have the ability to synthesise and export sugars into the grapevine system. The ratio of LA:Y is effectively that between the number of sources (exposed leaf area) and sinks(bunches) Post-fruitset, the developing fruit is the largest sink in the system (Mullins et al. 1992) and practices such as shoot trimming are employed to reduce competition, which is designed to encourage the transport of carbon to the developing fruit at the expense of vegetative growth (Williams 1996). Early in the growing season, the permanent structures of the grapevine provide the reserve carbohydrates necessary to sustain growth (Williams 1996).

Pinot Noir regularly elicits such phrases as ‘a notoriously fickle variety’ from some wine journalists (Robinson 1994) and as such intrigues growers and drinkers alike. This cool climate cultivar, especially when grown in warmer areas, is well known for a lack of colour density in wines (Jackson & Schuster 1987). The variety is responsible for the red wines of Burgundy and is one of the cultivars responsible for the sparkling wines of Champagne in France (Jackson & Schuster 1987, Jackson 2000). Jackson (2000) comments that under sub-optimal conditions, Pinot Noir produces wines lacking in varietal intensity, but under optimal conditions, the resulting wines are ‘aromatically distinctive’.

## 2.2 GRAPE COMPOUNDS

Water and sugar are the main components of berries (Hamilton & Coombe 2004, Jackson & Lombard 1993). In grape juice, 90 percent of soluble solids are sugar (predominantly glucose and fructose), whilst the remaining 10 percent consists of organic acids, phenolics, polysaccharides, pectins, potassium, proteins and other compounds (Hamilton & Coombe 2004). The amount of sugar in the grapes at harvest determines the potential alcohol content of wine.

Tartaric and malic acid are the two major acids found in grapes and make up over 90 percent of all acids, with citric and others making up the balance (Hamilton & Coombe 2004). Berry acidity is most often measured as titratable acidity (TA) usually in tartaric or sulfuric acid equivalents (Jackson & Lombard 1993). Phenolics are the third most significant group of compounds in red grapes (Jackson 2000) and are described here in detail due to their importance in red wine making. Phenolics are especially important to Pinot Noir wines as they contribute to the elegance, softness and depth for which Pinot Noir is renowned, setting these wines apart from other more full bodied varieties (Jackson & Schuster 1987).

### 2.2.1 PHENOLICS

Phenolics are compounds that are found in grapes and wine and contribute to colour, flavour and health benefits of wine (Kennedy et al. 2006, Mazza & Miniati 1993). Final concentrations of phenolics in grapes are influenced by viticultural and seasonal conditions (Kennedy 2008, Kennedy et al. 2006, Singleton 1988). There are two main groups of phenolics: flavonoids which are found in the solid parts of the grape and less commonly the bunch stem, and non-flavonoids that are found in the juice and pulp of grapes, the most abundant of which are hydroxycinnamic acids (Allen 1997, Kennedy et al. 2006, Somers & Verette 1988).

Hydroxycinnamic acids are important to white wine colour and are the most abundant of the phenolics in white wines but are present in similar amounts in red and white wines (Kennedy et al. 2006). The three most important groups of flavonoids in terms of sensory significance in *Vitis vinifera* grapes and wines are anthocyanins, flavan-3-ol monomers and proanthocyanidins all of which have the same base chemical structure (Kennedy et al. 2006, Souquet et al. 1996) (*Figure 2-2*) .

*Figure 2-2 (Flavonoid ring structure diagram from Jackson 2000 removed)*

#### *2.2.1.1 ANTHOCYANINS*

Anthocyanins contribute to the red colour of grapes and wine (Allen 1997, Kennedy et al. 2006, Mazza & Miniati 1993, Singleton 1988) and occur in *Vitis vinifera* in the stable 3-glucoside form (Singleton 1988). These glucosides are the result of conjugation between anthocyanidin subunits, such as cyanidin and malvidin, and glucose (Allen 1997, Jackson 2000). Anthocyanins are most commonly found in the skin of grapes (Kennedy et al. 2006), or more rarely, in 'teinturier' varieties such as Alicante Bouchet, in the flesh (Mullins et al. 1992).

Pinot Noir is unique as it is the only *Vitis vinifera* variety that contains only non-acylated anthocyanins (Fong et al. 1971, Mazza et al. 1999, Singleton 1988). The biosynthesis of anthocyanins occurs during berry ripening (Figure 2-3) following veraison, which is initially evident from the colour change of berries in red grape varieties from green to red (Herderich et al. 2004, Kennedy et al. 2006). There are two pathways for anthocyanin biosynthesis, regulated by the flavonoid 3'-hydroxylase (F3'H) or flavonoid 3'5'-hydroxylase (F3'5'H) enzymes (Boss et al. 1996). The F3'H enzyme regulates the pathway with end products cyanidin-3-glucoside and peonidin-3-glucoside and the F3'5'H enzyme regulates for petunidin-3-glucoside, delphinidin-3-gluoside and malvidin-3-glucoside (Boss et al. 1996).

*Figure 2-3 (Diagram of the biosynthesis of anthocyanins and phenolics and the maturation of tannins from flowering Ristic, 2004 cited by Herderich et al. 2004 removed).*

Exposure of developing berries, at veraison, has been shown to also stimulate anthocyanin synthesis, although the separation of light and temperature effects has proved difficult, with varying results from trials attempting to do so, as reviewed by Downey et al. (2006). Anthocyanins accumulate to a maximum point defined by Ribéreau-Gayon et al. (2000) as 'full ripeness' according to the sugar to acid ratio, after which anthocyanins begin to breakdown as grapes become 'overripe'. Full ripeness varies from region to region (Jackson & Lombard 1993) as do the rate of accumulation and final concentration of anthocyanins in the berries.

#### *2.2.1.2 FLAVAN-3-OL MONOMERS*

Flavan-3-ol monomers, such as (+)-catechin and (-)-epicatechin, contribute to the bitterness of wines and possibly astringency (Kennedy et al. 2006), but are colourless (Allen 1997). These compounds are the subunits that polymerise to form tannins (Allen 1997). Flavan-3-ols are synthesised predominately in seed coats (Downey et al. 2003) before veraison and are thought to change during veraison and berry ripening (Kennedy et al. 2006).

#### *2.2.1.3 PROANTHOCYANIDINS (CONDENSED TANNINS)*

Proanthocyanidins are flavonoid compounds consisting of polymers of flavan-3-ol subunits (Harbertson et al. 2008, Kennedy et al. 2006) generally created by the linking of repeating (-)-epicatechin subunits and terminating in a (+)-catechin unit (Singleton 1988). This resulting larger polyphenol is precipitable with protein (Singleton 1988). Proanthocyanidins are found in the skins and seeds of the berries and stems of bunches (Kennedy et al. 2006, Pastor del Rio & Kennedy 2006).

It is thought that proanthocyanidin biosynthesis occurs in the berry formation stage of berry development and that maturation occurs in the berry ripening stage (Figure 2-3) (Herderich et al. 2004). The amount of proanthocyanidins in berry skins is maximal at or near veraison and then is reduced by about 65 percent at harvest (Pastor del Rio & Kennedy 2006). It is thought that anthocyanin accumulation in the skins and proanthocyanidin accumulation in the seeds may well be linked (Darné, 1991, cited by Ribéreau-Gayon et al. 2000).

Pastor del Rio and Kennedy (2006) explain that the quantity and composition of proanthocyanidins are influenced by vineyard cultural practices but Harbetson et al. (2008) suggest that winemaking techniques most likely play a larger role. Proanthocyanidins found in skins and seeds differ in terms of their chemical makeup (Cortell et al. 2005) and perhaps the ratio between these two may influence ‘tannin ripeness’ perceived by winemakers (Herderich et al. 2004, Pastor del Rio & Kennedy 2006) as tannin derived from skins appears ‘riper’ and therefore more desirable than tannin derived from seeds (Kennedy 2008). Pinot Noir has been found to have a naturally high concentration of seed tannins (Kennedy 2008, Rustioni et al. 2009) with figures as high as 89 percent (Kennedy 2008) and 83 percent (Rustioni et al. 2009) of total grape tannin occurring as seed tannin reported.

## 2.3 WINE COMPOUNDS

The purpose of wine is to drink for enjoyment and as such there cannot be a replacement for the organoleptic experience and subjective assessment of wines, however wine analysis can deliver objective criteria against which to assess wines (Linskens & Jackson 1988). The sensory perception of wines can be altered by a range of factors, for example the colour of wine is affected by the pH of the wine, the amount of phenolic extraction achieved during winemaking and the age of the wine (Kennedy et al. 2006). The aroma of wine is associated with volatile compounds such as terpenes and methoxypyrazines which are found in grapes and wine (Jackson & Lombard 1993). The main differences between non-volatile sensory characteristics of red wines can be attributed to the variation in wine phenolics (Somers & Verette 1988).

### 2.3.1 PHENOLICS

Phenolic compounds are responsible for most of the fundamental sensory differences between wines including colour and flavour, however these compounds contribute little to the aroma of wines (Singleton 1988). It has also been reported that health benefits, such as cardiovascular disease prevention, are attributed to phenolic compounds, due to their antioxidant effects (Mazza & Miniati 1993). The total amount of phenolics in wine is not only influenced by the amount in the grapes, but also winemaking processes such as maceration, fermentation time and use of oak barrels (Kennedy et al. 2006, Mazza et al. 1999, Singleton 1988). However, the amount of phenolics extracted into wine is always less than the amount of phenolics in the grapes as reportedly less than 50 percent is extracted (Somers & Verette 1988) with factors such as pH, ethanol and sulphur dioxide also affecting phenolic extraction (Jackson 2000).

#### *2.3.1.1 ANTHOCYANINS*

Anthocyanins are compounds found in grapes which contribute to wine colour, both independently and as they interact with colourless phenolics to undergo the copigmentation process (Cortell 2006, Mazza et al. 1999). These compounds are extracted during the crushing, maceration, pressing and fermentation stages of winemaking (Mazza et al. 1999). Anthocyanins are susceptible to degradation within the wine solution and also to bleaching as

a result of sulphur dioxide additions used in winemaking (Kennedy et al. 2006, Smith et al. 2007). Other compounds such as acetaldehyde can increase the colour of wines, through the sequestration of sulphur dioxide (Singleton 1988).

*Figure 2-4 (Diagram of the forms of anthocyanins and the equilibrium in which they exist at differing pH levels from Jackson 2000 removed).*

Anthocyanins exist in a variety of equilibrium forms in wine, mainly dependent on pH and the presence of sulphur dioxide (Kennedy et al. 2006, Mercurio et al. 2007, Singleton 1988). The flavylium form is the only form that appears red, the quinoidal base appears violet, and the flavene sulfonate and carbinol base are colourless (Singleton 1988) (Figure 2-3). The carbinol base also exists in equilibrium with the yellow-coloured chalcone form (Singleton 1988). At wine pH, as little as 10 percent of anthocyanins exist in the flavylium form (Kennedy et al. 2006), but decreasing the pH increases this percentage (Mercurio et al. 2007). Anthocyanins can be incorporated into pigmented polymers to stabilise the ionised flavylium form, which has an amber-red appearance (Singleton 1988).

Acylation of anthocyanins has been suggested to increase red wine colour density due to stabilisation of the flavylium form of anthocyanins (Allen 1997). However, Pinot Noir wines contain only non-acylated anthocyanins, delphinidin 3-monoglucoside, cyanidin 3-monoglucoside, petunidin 3-monoglucoside, peonidin 3-monoglucoside and malvidin 3-monoglucoside (Mazza et al. 1999, Singleton 1988).

#### *2.3.1.2 FLAVAN-3-OL MONOMERS*

Flavan-3-ols contribute to both bitterness and astringency in wine (Kennedy et al. 2006). Astringency, which causes mouth puckering or drying (Gawel 1998), can be an undesirable trait in wines, but in the presence of other compounds can improve wine texture (Kennedy et al. 2006). Gawel (1998) suggests that individuals perceive astringency differently in terms of

intensity, persistence and quality. The concentration in wine of flavan-3-ols is increased by longer extraction times, higher temperatures and higher alcohol (Kennedy et al. 2006).

#### *2.3.1.3 PROANTHOCYANIDINS (CONDENSED TANNINS)*

Proanthocyanidins, condensed tannins or polymeric flavan-3-ols are extracted from bunch stems, berry skins and seeds during the fermentation and maceration stages of winemaking (Pastor del Rio & Kennedy 2006). Tannins also play a significant role in the development of colour in wine as anthocyanins react with the tannins to form pigmented polymers also known as polymeric pigments or pigmented tannins (Somers 1971, cited by Smith et al. 2007). The chains of flavan-3-ols that make up proanthocyanidins must be of an adequate size to form stable bonds with proteins, but must not be so bulky as to prevent bonding by limiting access to the protein site (Singleton 1988). When proanthocyanidins become too bulky, they precipitate out of the wine solution (Singleton 1988).

During winemaking, the proanthocyanidins are extracted from the grape skins during fermentation and maceration (Pastor del Rio & Kennedy 2006, Somers & Verette 1988). Seed proanthocyanidins are extracted over a longer period as the seed cuticle must first be dissolved by the ethanol produced from fermentation with further extraction occurring through the post fermentation stage of winemaking (Ribéreau-Gayon et al. 2000). Pinot Noir wines are lower in tannin than other red varietals due to the high ratio of seed (89 percent) to skin (11 percent) tannin in the grapes and the difficulty in their extraction (Kennedy 2008). Kennedy (2008) found only 6 percent of seed tannins were extracted into the wines, whereas 29 percent of skin tannin was extracted with a resulting ratio of 36 percent skin tannin in the wines and 64 percent seed tannin.

#### *2.3.1.4 PIGMENTED POLYMERS*

The reaction between anthocyanins and other phenols such as proanthocyanidins produces pigmented polymers, which are significantly more stable than other phenolic compounds such as free anthocyanins, and are therefore of major importance to the long-term colour stability of red wines (Somers & Evans 1977). Pigmented polymers are more resistant to free sulphur dioxide (Allen 1997), oxidation (Jackson 2000) and changes in pH (Mercurio et al. 2007) than free anthocyanins. Pigmented polymers not only contribute to colour stability of wines, but

also play a role in the modification of flavour and astringency (Kennedy et al. 2006). It is ideal if polymerisation can occur during wine maturation as free anthocyanins are susceptible to irreversible degradation (Jackson 2000).

Allen (1997) suggests that if red wines are fermented under controlled conditions, then a slow polymerisation process will develop more complex and stable forms of anthocyanins. Gao et al. (1997) found that temperature was the critical factor for development of polymeric pigments in Pinot Noir wines. However, if this polymerisation process is too extensive, then long chain polymers may result, which can result in the precipitation of the red pigment from the wine (Allen 1997).

## 2.4 WINEMAKING

The winemaking process consists of a number of variables that can be manipulated to produce a desired wine style, for example fermentation temperature and time and skin contact time (Boulton 2001, Gao et al. 1997, Mazza et al. 1999, Mazza & Miniati 1993). These factors together with wine chemistry phenomena such as ionisation of anthocyanins and polymerisation with other compounds to form stable colour, means that there is often not a very good correlation between grape and wine colour (Smith et al. 2007).

Different winemaking practices result in different degrees of extraction of anthocyanin and phenolic compounds therefore influencing the resulting wines (Kennedy et al. 2006, Mazza et al. 1999, Singleton 1988). During wine ageing there is greater 'quantitative and qualitative changes in the concentration and structure of phenolics … than in almost any other wine constituent' (Jackson 2000).

The ageing of wines, in tank, barrel or bottle, produces further reactions that influence the final composition of wines. Maturation in oak barrels results in extraction of further phenolics from the oak which are 'not flavanoids and a large portion are hydrolysable tannins not otherwise found in wine' (Singleton 1988). Caftaric acid and free anthocyanins are incorporated into polymers during the ageing process (Singleton 1988) and the tannin transformations mentioned previously not only assist in colour modification, but also the softening of flavours and astringency reduction. As these phenolic compounds are

incorporated and modified, the sensory properties of the wines are altered and the ability to manage these changes is a desire of winemakers (Kennedy et al. 2006).

## 2.5 WINE COPIGMENTATION

Copigmentation is a non-covalent interaction (Kennedy et al. 2006) that occurs in wines when 'pigments and other non-coloured organic components form molecular associations or complexes' (Boulton 2001). The review by Boulton (2001) highlights a gap in traditional wine colour measurements (Somers & Evans 1974, Somers & Evans 1977), which usually do not take this phenomenon into account. The reaction between non-coloured phenolics and anthocyanin glucosides can account for up to 50 percent of the colour in young wines (Boulton 2001). This is why colour pigments can display far greater colour in wines than is measured by their concentration (Boulton 2001, Kennedy et al. 2006). Copigmentation increases the amount of anthocyanins present in the flavylium form than would be expected at a given wine pH (Kennedy et al. 2006).

The difficulty in studying the phenolic reactions in wine is complicated by the wide variety of potential substrates available in the solution. Some of these substrates, such as acetaldehyde, are the result of alcoholic fermentation (Allen 1997, Mazza & Miniati 1993) and other compounds such as sulphur dioxide and oxygen are also known to affect the copigmentation process (Mazza & Miniati 1993). Anthocyanins are present in the coloured flavylium cation form in more acidic solutions such as the low pH of wines, so copigmentation most commonly involves these forms (Kennedy et al. 2006). Ethanol, a product of fermentation, appears to play an important role in copigmentation as it is seems to act against this process (Kennedy et al. 2006) as exemplified by the reduction in colour of wines during fermentation (Somers and Evans 1979, cited by Kennedy et al. 2006).

## 2.6 WINE ANALYSES

The development of wine analysis indices by Somers and Evans (1974) and (1977) enabled the measurement of wine colour and other colour development factors such as ionisation of anthocyanins and formation of stable forms of colour (Mercurio et al. 2007). The original method analysed the wine in its natural state without adjustment, so comparison between

different wines could identify differences due to pH and alcohol effects. The 'Somers assay' was recently modified (Mercurio et al. 2007) to standardise wine samples before analysis to a pH of 3.4 and alcohol concentration of 12 % v/v, through dilution in a buffer solution .

Colour density is the most commonly used wine colour index is as it gives a quantifiable measure (Mercurio et al. 2007) to the initial sensory perception experienced by consumers. In some varieties, such as Cabernet Sauvignon and Shiraz, wine colour has been shown to be well correlated with wine quality ratings (Somers & Evans 1974), however colour 'gives only a rough indication of grape pigmentation, the duration of skin contact, probable wine age, and the presence or absence of a few wine faults' (Jackson 2000). The colour density measurement was modified by Mercurio et al. (2007) to be conducted under excess acetaldehyde conditions so that the effect of varying $SO_2$ concentrations in the wines could be eliminated through the formation of $SO_2$-acetaldehyde adducts. Any anthocyanins bleached from $SO_2$ additions after fermentation revert to the free form that can form the coloured flavylium ion (Mercurio et al. 2007, Somers & Evans 1977).

Wine hue is another index used to quantify the appearance of wine and is the ratio between absorbance at 420nm and 520nm of wine (Sudraud 1958, cited by Somers and Evans 1977). The 520nm wavelength represents the green part of the colour spectrum and a higher absorbance at this wavelength will result in greater transmission of light deficient in green and therefore appears red (Allen 1997). The 420nm wavelength, is near the ultraviolet end of the colour spectrum and results in greater transmission of light deficient in violet and therefore appears yellow/orange (Mercurio et al. 2007). Hue values are inversely related to colour density (Mazza et al. 1999) and increase with age (Somers & Evans 1977).

The degree of ionisation of anthocyanins 'is intended to mean the percentage of total anthocyanins which are in the colour or flavylium form' (Somers & Evans 1974). This measure is achieved by calculating the percentage ratio between anthocyanins in the flavylium form at wine pH and at pH<1.0 (Somers & Evans 1974). The flavylium ion is favoured under acidic conditions (Singleton 1988). Winemaking deficiencies or faults in red wines can possibly show through as very low values for this parameter (Somers & Evans 1977). The degree of

ionisation of anthocyanins is also influenced by pH and $SO_2$ level (Somers & Evans 1977), with these effects removed in the modified Somers method (Mercurio et al. 2007).

Somers and Evans (1977) developed two indices that quantify the rate of ageing, chemical age 1 and chemical age 2. The rate of ageing is indicated as the stage of conversion process of anthocyanins to polymeric pigments and this value increases with the ageing of wines (Somers & Evans 1977). $SO_2$ resistant pigment is another measure arising from the modified Somers measurements (Mercurio et al. 2007). It has been shown to correlate well with pigmented polymer concentration as analysed by HPLC (Peng et al. 2002).

Chemical age 1 is taken at standard wine pH and is the ratio between the absorbance at 520nm in the presence of $SO_2$ and absorbance at 520nm in the presence of acetaldehyde, so indicates the ratio of pigmented polymer to colour in the absence of $SO_2$ bleaching (Mercurio et al. 2007, Somers & Evans 1977). Chemical age 2 uses the ratio of $SO_2$ resistant colour (520 nm) and colour after a hydrochloric acid dilution. Pigmented polymers are resistant to $SO_2$ bleaching and anthocyanins are fully ionised at low pH (Somers & Evans 1974), so chemical age 2 estimates the proportion of colour as pigmented polymers, compared with the 'total' colour (pigmented polymers plus anthocyanins).

The dilution of the wine with reagents modifies the original Somers measures thereby altering the copigmentation phenomenon of wine (Mercurio et al. 2007). Boulton (2001) comments that this results in a deviation from Beer's law, however Mercurio et al. (2007) consider that the standardisation of pH and alcohol level minimises these effects.

## 2.7 EXPERIMENTAL APPROACH

There is continuing debate as to what constitutes the best vineyard management practices to manipulate yield in Pinot Noir and whether higher yields mean lower quality (Jackson 1987). Juice composition has often been studied (Reynolds et al. 1994, Reynolds et al. 1996, Sommer & Clingeleffer 1993) as a proxy for wine quality, but there is little in the literature that evaluates viticultural trials by making wine from treatments. The current study was designed to investigate the yield and quality relationship in Pinot Noir not only in terms of yield and yield components, but also in terms of grape and wine composition. The common

commercial Pinot Noir cool climate vineyard management practices of winter pruning, bunch removal, leaf removal and shoot trimming were examined over three consecutive seasons. Figure 2-5 shows when, during the berry growth cycle, vineyard management practices occur in relation to physiological berry development. Grape composition was analysed at harvest and small scale, standard protocol winemaking was undertaken to enable analysis of vineyard influences in the finished wines.

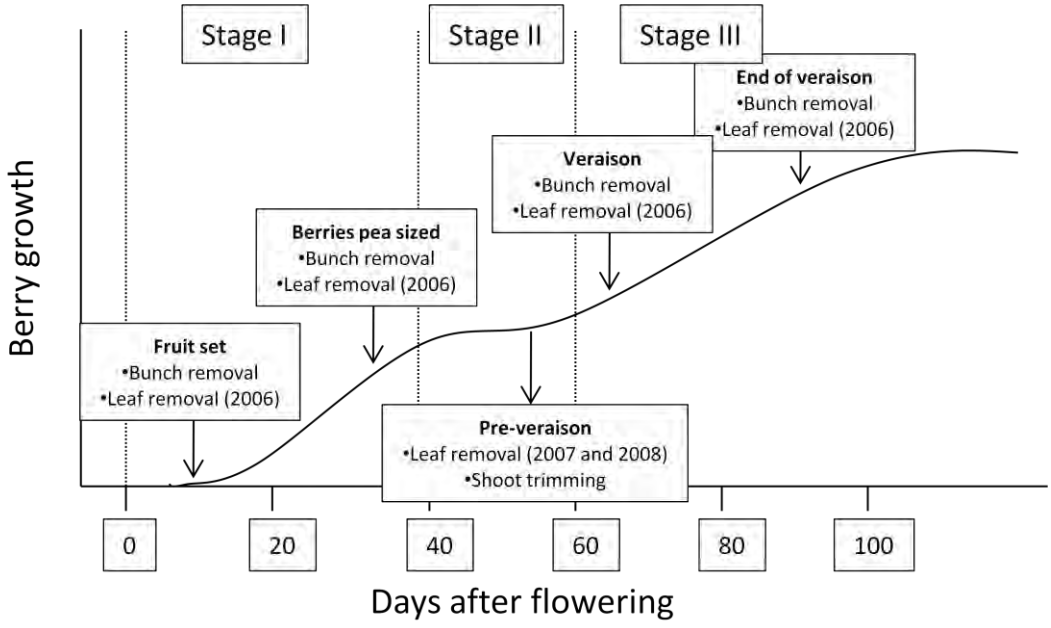


**Figure 2-5 Summary of vineyard management practices investigated in the current study and timing of these interventions during the berry growth cycle.**

# CHAPTER 3: SITE DESCRIPTION AND GENERAL MATERIALS AND METHODS

## 3.1 SITE ANALYSIS

### 3.1.1 LOCATION

Trials were conducted in northern Tasmania, Australia in the Tamar Valley wine region (Figure 3-1) at a commercial vineyard (Figure 3-2). This vineyard is situated at latitude 41.2 °S, longitude 146.9 °E with an altitude of 45 m. A relatively homogeneous vineyard block of moderate vigour was selected for the purpose of a trial site. The block was selected as homogeneous by using normalised difference vegetative index (NDVI) aerial maps flown at veraison during the 2004-2005 season (Figure 3-3). Uniform vigour across the trial block was desirable in an attempt to eliminate vigour effects which have been shown to have a considerable impact on fruit and wine composition (Cortell 2006, Cortell et al. 2007a, Cortell et al. 2007b).

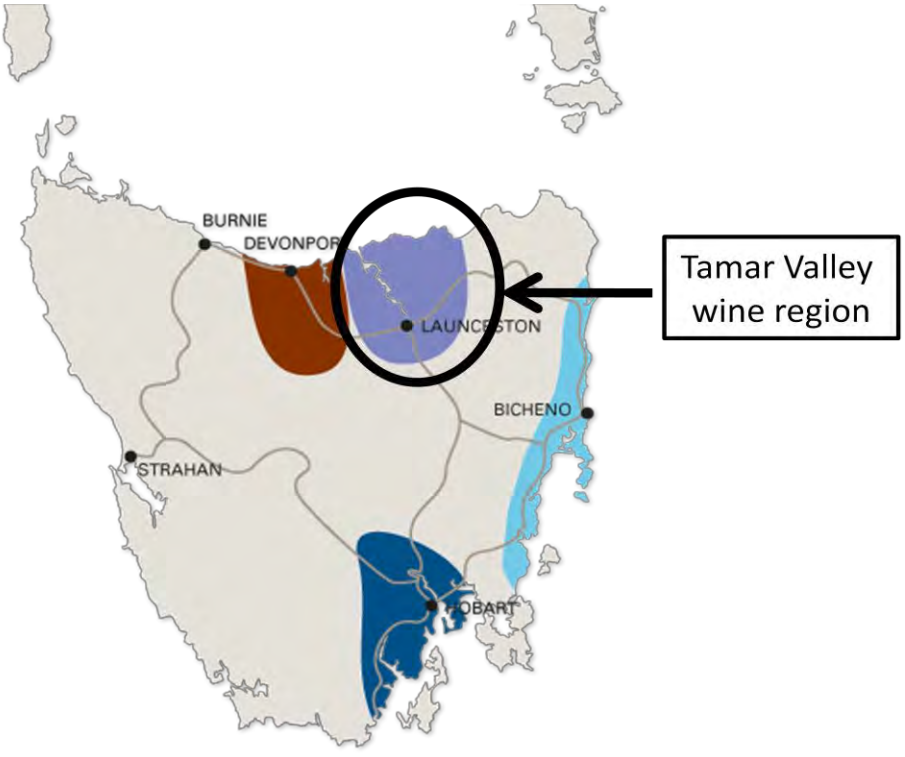


**Figure 3-1 The four main wine growing regions of Tasmania, Australia. Circled is the Tamar Valley wine region where the experimental vineyard was located.**

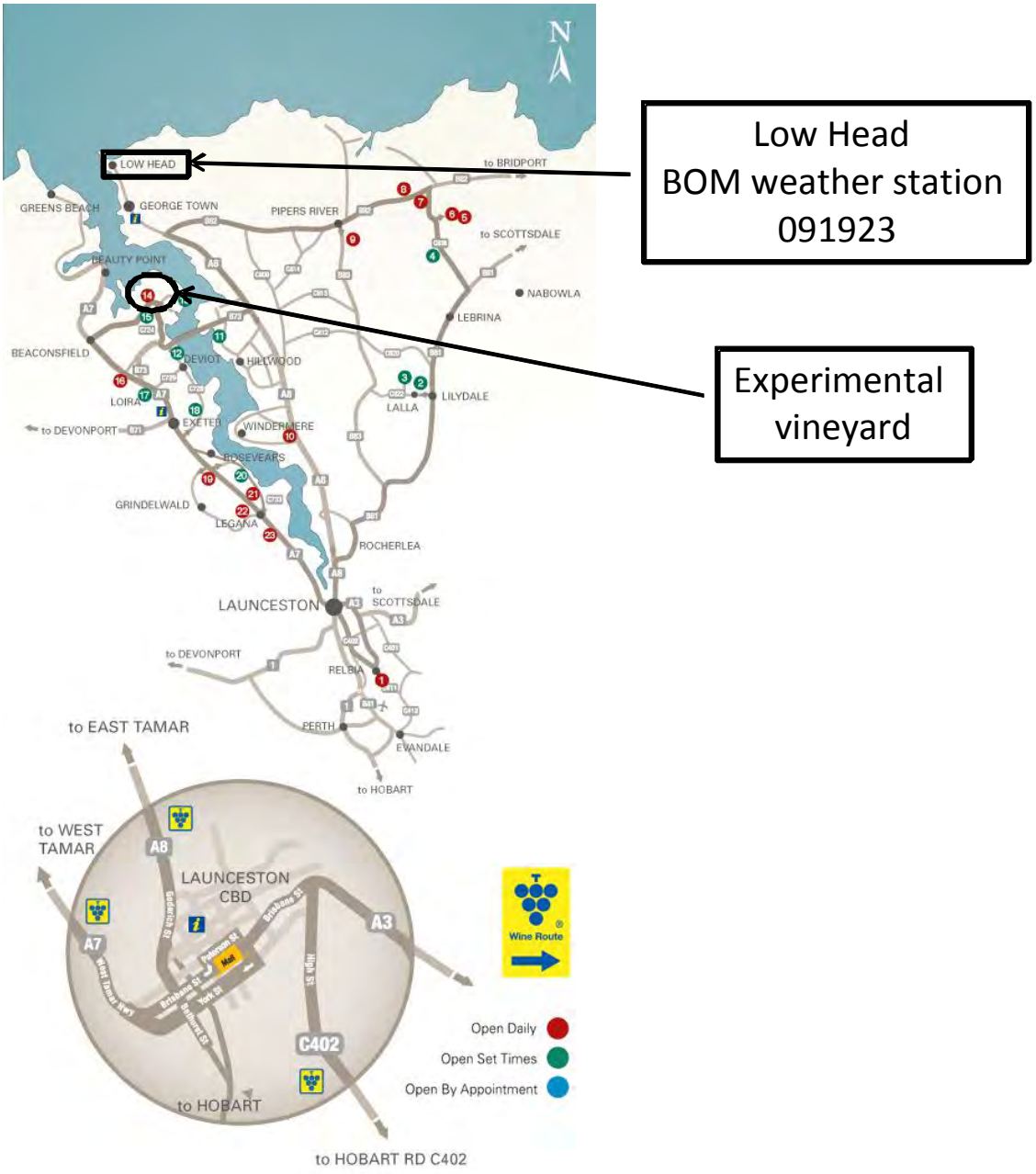


**Figure 3-2** **Tamar Valley wine region in Tasmania. The circle shows the location of the experimental vineyard and the rectangle shows the Low Head Australian Bureau of Meteorology (BOM) weather station (091923).**

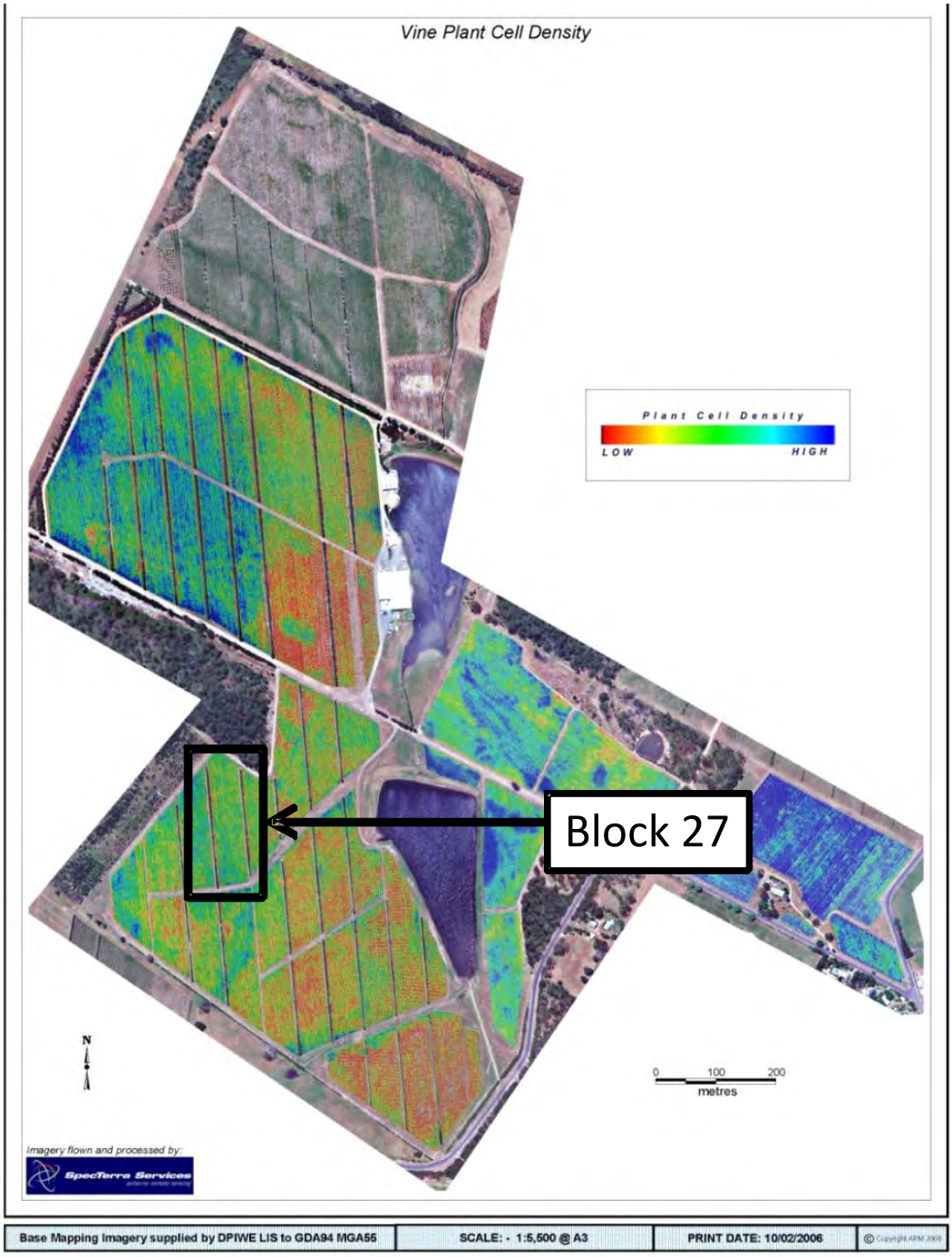


**Figure 3-3** **Normalised difference vegetative index (NDVI) map of the experimental vineyard (flown at veraison in 2006) extrapolated to vine plant cell density to determine vigour zones. Highlighted is experimental Block 27, chosen for its homogeneity and moderate vigour.**

### 3.1.2 SEASONAL WEATHER CONDITIONS

The nearest Australian Bureau of Meteorology (BOM) weather station to the experimental vineyard was the Low Head weather station (BOM station: 091293) located on the eastern mouth of the Tamar River (Figure 3-2). Three basic seasonal weather condition variables were selected from this station to that could be easily accessed from the BOM website (www.bom.gov.au), namely daily maximum temperature, daily minimum temperature and daily rainfall. From these three data sets, mean January temperature (MJT), mean February temperature (MFT), growing season rainfall (Rain Sep-May), annual rainfall (Rain Jul-Jun), degree days up the end of flowering (DD Sep-Dec) and growing season degree days (DD Sep-May) were calculated (Table 3-1).

**Table 3-1 Low Head weather station data for the three experimental seasons of the current study and the mean of the previous 10 years (BOM station: 091923).**

| | 2006 | 2007 | 2008 | **10 year mean (1998-2008)** |
|---|---|---|---|---|
| Mean January temperature (°C) | 17.2 | 17.5 | 18.1 | **17.2** |
| Mean February temperature (°C) | 17.7 | 19.3 | 17.4 | **17.8** |
| Rain (mm) (Sep-May) | 532.4 | 316.6 | 378.2 | **430.0** |
| Rain (mm) (Jul-Jun) | 752.8 | 390.4 | 561.2 | **648.3** |
| Degree Days (Sep-May) (base 10 °C) | 1247.6 | 1364.4 | 1358.6 | **1272.5** |
| Degree Days (Sep-Dec) (base 10 °C) | 486.4 | 371.5 | 467.9 | **421.2** |

Data from the Low Head weather station showed a 10 year mean January temperature (MJT) of 17.2 °C, which is well below the Smart and Dry (1980) cool climate classification of less than 19.0 °C MJT. Mean monthly maximum and minimum temperatures calculated from data from the Low Head weather station are presented in Figure 3-4. This figure highlighted the low maximum daytime temperatures, which would most likely be photosynthesis limiting.

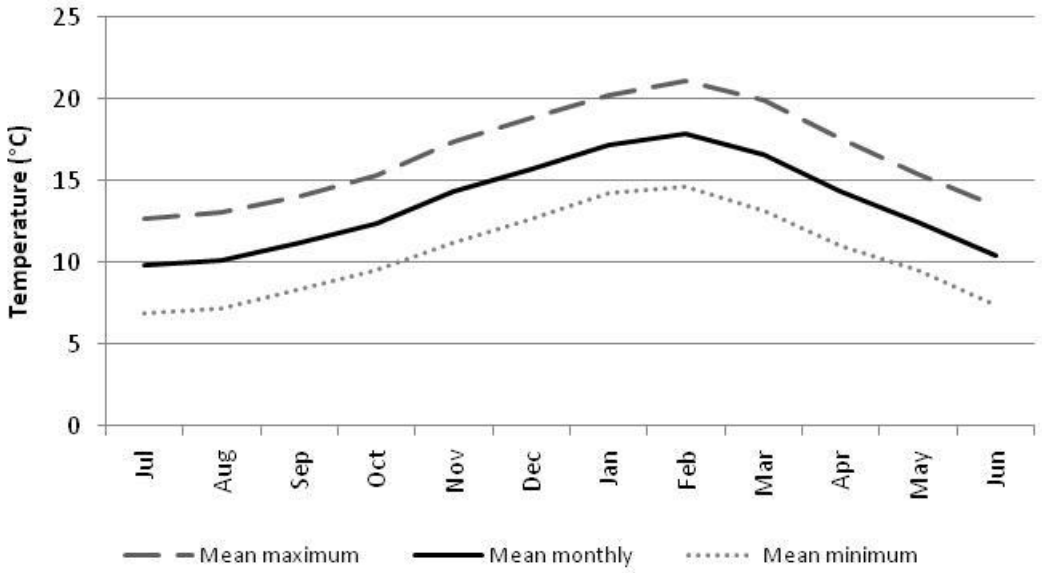


**Figure 3-4 Mean monthly average, maximum and minimum temperatures for Low Head (BOM station: 091293). Data are means of 13 years data (1998-2010).**

Mean January temperature (MJT) and mean February temperature (MFT) data for the experimental seasons are presented in Figure 3-5 as a deviation from the 10 year mean (1998 – 2008). The x axis represents the 10 year mean and columns above this axis show a warmer than average month during the corresponding season and columns below this axis show a cooler than average month. The MJT and MFT data have been displayed as these are the month prior to and month of veraison respectively in Tasmania and therefore are of importance to berry ripening (Mullins et al. 1992). The 2006 season was close to the 10 year mean for both MJT and MFT. The 2007 season was warmer for MJT and much warmer for MFT (19.3 °C) than the 10 year mean (17.8 °C). The 2008 season had a warmer than average MJT, but cooler than average MFT.

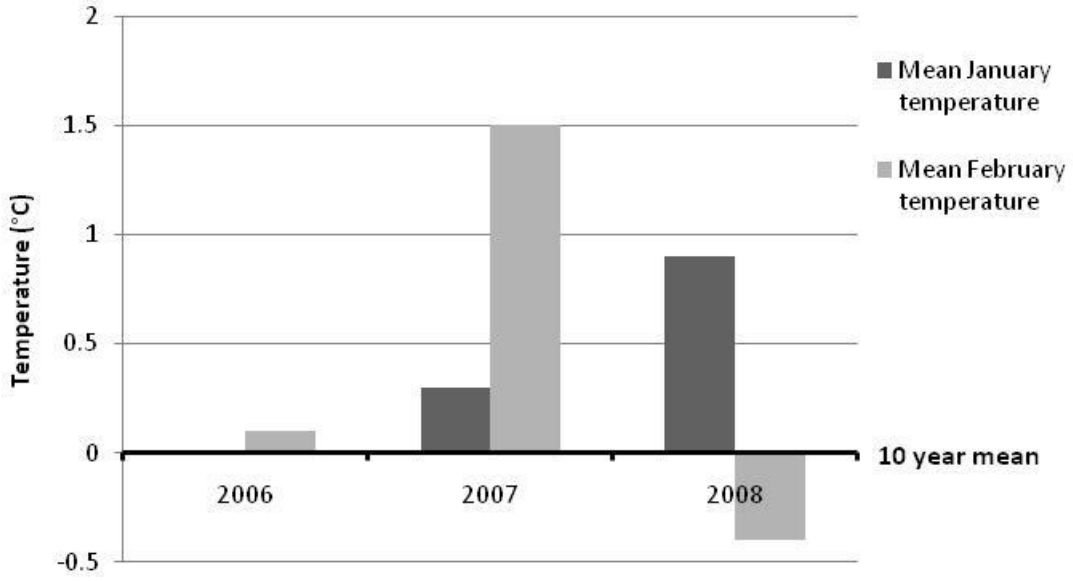


**Figure 3-5 Mean January temperature and mean February temperature at the Low Head weather station (BOM station: 091923). The x axis represents the 10 year mean (1998 – 2008) and columns for each season represent the deviation from the 10 year mean.**

The growing season for the current study was defined by the author to be from September 1st to May 31st, as budburst usually occurs during September and harvest for Pinot Noir can occur as late as May in Tasmania. Cumulative daily degree days were calculated for this period (Figure 3-7) by using 10 °C as a base figure and then taking the daily average temperature and subtracting 10 °C (as shown below) (Amerine & Winkler 1944). Calculation of degree days uses a base figure of 10 °C as it has been shown that below this temperature very little vine growth occurs (Amerine & Winkler 1944). Total degree days up to the end of flowering and total growing season degree days are displayed in Figure 3-6 as deviations from the 10 year mean. Representation of data in this way shows that the 2006 season had a warmer than average period up to the end of flowering, but a cooler than average total growing season degree day accumulation. The 2007 season was cooler than average up to the end of flowering with warmer than average growing season degree days. The 2008 season was warmer than average both up to the end of the growing season.

**Degree day:**

$$\frac{(T_{max} + T_{min})}{2} - 10$$

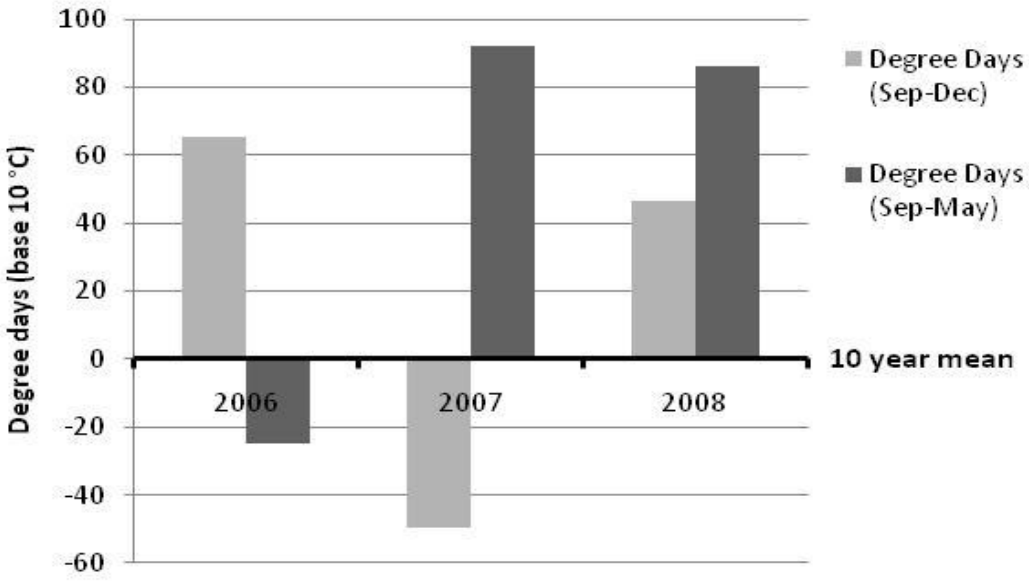


**Figure 3-6 Total growing season degree days (Sep-May) and total degree days up to the end of flowering (Sep-Dec) at the Low Head weather station (BOM station: 091923). The x axis represents the 10 year mean (1998 – 2008) and columns for each season represent the deviation from the 10 year mean.**

Although total growing season degree days is a common parameter to define heat accumulation within a season, plotting the deviation from the 10 year mean of the daily accumulation of degree days provides more of a picture as to how the season has progressed (Figure 3-7). A seasonal line progressing in parallel to the x axis, or the 10 year mean, indicates an average daily accumulation of degree days for that season. An upward slope of the seasonal line indicates an above average period of warmth and a downwards slope of the seasonal line indicates a cooler than average period. For example total growing season degree days of the 2006 season (1247.6) is close to the 10 year mean (1272.5), however Figure 3-7 diagrammatically shows an average progression of the 2006 season up until mid-October after which there was a warmer than average period until the beginning of December, after which degree day accumulation was around average until the beginning of April when there was a decline in degree day accumulation.

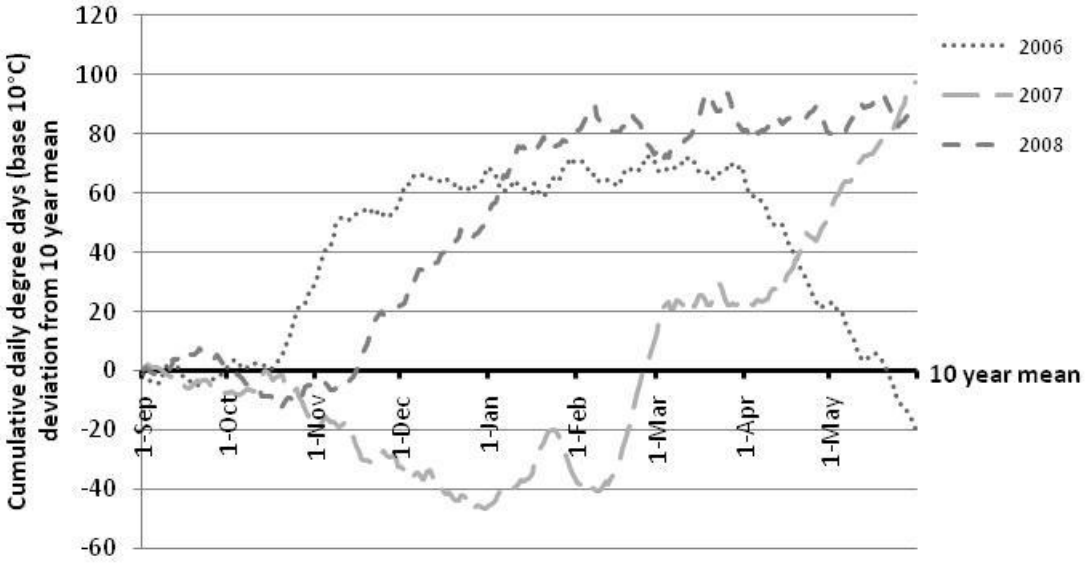


**Figure 3-7 Cumulative daily degree days deviation from the 10 year mean for seasons 2006, 2007 and 2008 for Low Head (BOM station: 091293). The x axis represents the 10 year mean (1998 – 2008) and lines for each season represent the deviation from the mean.**

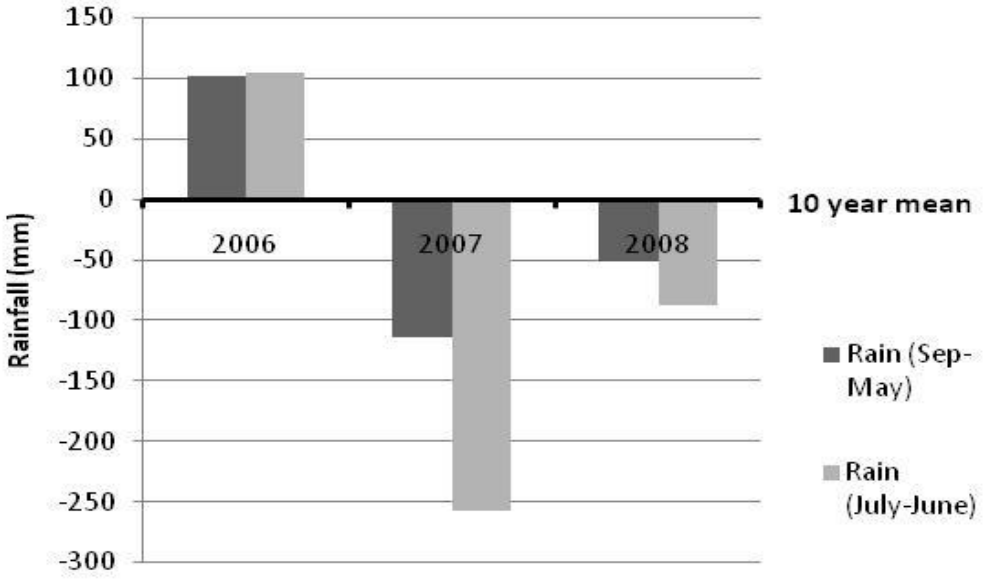


**Figure 3-8 Growing season rainfall (Sep-May) and annual rainfall (July-June) at the Low Head weather station (BOM station: 091923). The x axis represents the 10 year mean (1998 – 2008) and columns for each season represent the deviation from the 10 year mean.**

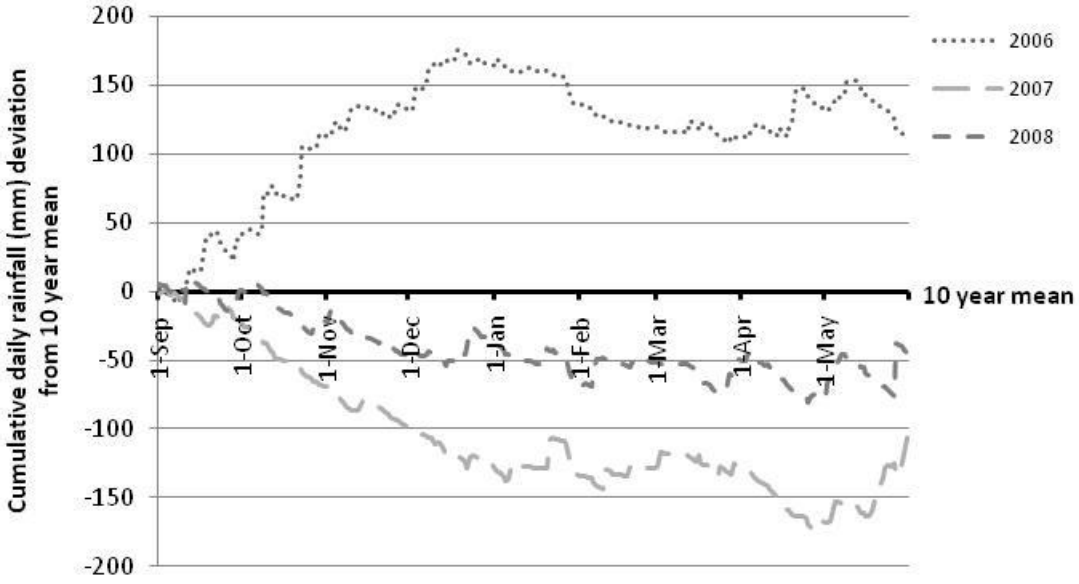


**Figure 3-9 Cumulative daily rainfall deviation from the 10 year mean for seasons 2006, 2007 and 2008 for Low Head (BOM station: 091293). The x axis represents the 10 year mean (1998 – 2008) and lines for each season represent the deviation from the mean.**

Data for annual and growing season rainfall is presented as total rainfall deviation from the 10 year mean (Figure 3-8). This figure indicates the higher than average rainfall during the 2006 season and lower than average rainfall for both the 2007 and 2008 seasons. The daily accumulation of rainfall is presented in Figure 3-9 which indicates, for example, the wetter than average spring period during the 2006 season.

### 3.1.3 SOILS

Soils of the trial site were formed on Tertiary sediments and consist of bleached sandy topsoil overlying orange and grey mottled clayey subsoil (S. Rees pers. comm.). According to Isbell (1996) they are classified as Bleach-Mottled, Natric, Brown Kurosols, indicating strongly acid subsoils ($pH_w < 5.5$) and the major part of the upper 0.2 m of the $B_2$ horizon is sodic (an exchangeable sodium percentage (ESP) of > 6 in the upper 20 cm subsoil).

### 3.1.4 PLANT MATERIAL

A block of clone 114 (8418) Pinot Noir vines, planted in 2000 on own roots, were used for the current study. Clone 114 is an early ripening, moderately vigorous clone (R. Smart pers. comm.). Vines in this block, according to the yield to pruning weight ratio, were of moderate vigour as values before the commencement of the current study fell in the range of 5-10 (Smart & Robinson 1991).

Planting density was 2963 vines per hectare with a row spacing of 2.25 m and a vine spacing of 1.5 m and a north to south row orientation. The experimental block was trained to a Scott Henry trellis system (Freeman et al. 1988, Smart & Robinson 1991, Smart 1988) and had two fruiting wires at 1 m and 1.15 m from the ground. Vines had a crown just below the bottom fruiting wire and had previously been pruned to an average total of 35 nodes per vine (23 nodes/metre) over 4 canes. The experimental vineyard used a combination of soil moisture probes and vine observation to monitor irrigation. The irrigation rationale was to stop shoot growth prior to veraison, and retain a non-water stressed, healthy canopy through the ripening phase.

## 3.2 GENERAL MATERIALS AND METHODS

The following materials and methods apply to all the experimental chapters (Chapters 4-7). Further detail is given in individual chapters for specific materials and methods specific to that chapter. In Tasmania, the annual growth cycle of *Vitis vinifera* grapevines commences in September and concludes between March to May at harvest. For the current study, the year in which the grapes were harvested is the year that will be used to name the season, i.e. the 2005-2006 season is referred to as the 2006 season.

### 3.2.1 YIELD AND YIELD COMPONENTS

Yield (using scales accurate to 0.01 kg [A&D Co. Ltd., Korea, SK-20K]) and bunch number were recorded at harvest and average bunch weights recorded. Bunch number at harvest and node number from pruning at the beginning of the season was used to calculate bunches per node. Before pruning, canes were counted and at pruning the pruned canes were weighed. Mean cane weight and yield to pruning weight ratio were calculated. The yield to pruning weight

ratio correlates well with leaf area to yield which is a common, but destructive measure of vine balance (Gal et al. 1996).

In 2007 and 2008, 100 berry samples were taken the day before harvest, weighed and berry weight calculated. Berries were sampled from the top, the middle and the bottom of randomly selected bunches on the external and then the internal side of bunches and from both sides of the trellis. In 2008, a season of high *Botrytis cinerea* pressure, the percentage of bunches affected was recorded at harvest by separate harvesting and weighing of affected bunches.

### 3.2.2 FRUIT COMPOSITION

#### *3.2.2.1 GRAPE COMPOSITION*

In 2006 and 2007, all treatments were harvested on the same day. Date of harvest was initially to be driven by sugar maturity of the grapes, with harvest at around 13 °Be. However, in the period of March 4$^{th}$ to April 4$^{th}$ in 2006, there were 12 rain days totalling 48 mm rainfall and in light of increased disease pressure due to ideal *Botrytis cinerea* conditions the previous spring, it was decided to commence harvest as soon as possible. Space in the microwinery allowing, fruit was harvested as quickly as possible. A similar situation developed in 2007, with 38 mm of rain falling over 6 days in the month leading up to the commencement of harvest and again disease pressure determined harvest date. Rainfall was lower in the 2008 season, but attempts once again to try and pick fruit at 13 °Be sugar resulted in a higher incidence of *Botrytis cinerea* in the fruit harvested and thus the percent of fruit infected was recorded for this season only.

Trial maturity was determined from overall block maturity samples taken by the commercial vineyard were the trials were situated to track maturity to around 11.5-12.0 °Be and then individual treatment 100 berry samples within each trial were taken to determine sugar maturity. It was taken into account that berry samples often show a higher sugar content than must (Hamilton & Coombe 2004).

Total soluble solids (TSS), pH and titratable acidity (TA) in 2006 were analysed from 100 berry samples taken on the day of harvest from each of the four replicates. Samples were hand

crushed in plastic bags and juice strained and analysed. In 2007 and 2008 seasons, must samples were taken from the Baesso 80 kg crusher/destemmer unit (Australian Winemakers, Victoria, Australia). TSS was analysed using a handheld refractometer (Vintessential manufactured product FG103/113, China, accurate to 0.2 percent with auto temperature compensation [ATC]) to measure °Brix, which was expressed as °Baume (°Be) (°Brix ÷ 1.8) (Hamilton & Coombe 2004). pH and TA were analysed using a 785 DMP Titrino autotitrator (Metrohm, Switzerland) with a 728 magnetic stirrer attached (Metrohm, Switzerland).

In all three seasons, a second 100 berry sample was taken the day of harvest and frozen at -20 °C for later analysis of grape total anthocyanins according to the Australian Wine Research Institute (2006), which is a modification of Iland (2000). For the 2007 and 2008 seasons, grapes were weighed fresh for berry weight data and then frozen at -20 °C for later analysis of total phenolics as per Iland (2000) in addition to total anthocyanins (Australian Wine Research Institute 2006). In 2006, an Ultra-Turrax (IKA, Germany) was used to homogenise the grapes and in 2007 and 2008, a Waring 8610EG blender (Waring Commercial, USA) was used and each sample was homogenised for 30 seconds. Differences between these homogenisation methods has previously been shown to not affect spectroscopic analyses (Cynkar et al. 2004). Homogenates were weighed using FX300i laboratory scales, accurate to 0.001 g (A&D Co. Ltd., Korea).

Samples were centrifuged (Centurion Scientific EB Series E25B5 [Centurion Scientific, United Kingdom]) and sample absorbance read (Metertech UV/Vis SP8001 spectrophotometer [Metertech, Taiwan]). For total milligrams of grape anthocyanins per gram of fresh fruit weight, the absorbance of samples were read at 520 nm ($A_{520}$) (Australian Wine Research Institute 2006). For total grape phenolics expressed as absorbance units (AU) per gram of fresh fruit weight, the absorbance of samples were read at 280 nm ($A_{280}$) (Iland 2000).

**Grape total anthocyanins (mg/g):**

$$\frac{A_{520} \text{ x } 20 \text{ x final extract volume (mL) x } 1000}{500 \text{ x } 100 \text{ x homogenate weight (g)}}$$

**Grape total phenolics (AU/g):**

$$\frac{A_{280} \text{ x } 20 \text{ x (final extract volume (mL)/100) x (50 x mean berry weight (g)/homogenate weight (g)) x (1/50)}}{\text{Mean berry weight (g)}}$$

#### *3.2.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)*

Previous studies by Mazza *et al.* (1999) reported only five nonacylated anthocyanins in Pinot Noir, delphinidin 3-monoglucoside (delphinidin), cyanidin 3-monoglucoside (cyanidin), petunidin 3-monoglucoside (petunidin), peonidin 3-monoglucoside (peonidin) and malvidin 3-monoglucoside (malvidin). To determine the concentration of these five anthocyanins, grape homogenate extract supernatants were analysed using a Waters Alliance 2690 HPLC system equipped with a Waters 996 photodiode array detector. Separation was achieved on a Waters Xterra C18 column (5 µm, 4.6 x 250 mm). A flow rate 1 mL/min was used. Solvent A was methanol, and solvent B was 5 percent (v/v) formic acid in water. For the homogenate extracts an isocratic method of 22 percent solvent A and 78 percent solvent B was used with a run time of 30 minutes. A 25 µL sample was injected directly into the HPLC. Wavelengths from 200-600 nm were monitored, and chromatograms were generated at 520 nm as adapted from Heazlewood (2005). Results from HPLC analysis were reported as malvidin-3-glucoside equivalents.

### 3.2.3 WINE COMPOSITION

#### *3.2.3.1 WINEMAKING*

Small scale winemaking was utilised to investigate differences between treatments applied in the vineyard rather than to try and replicate commercial winemaking practices (Sampaio et al. 2007). All bunches infected by *Botrytis cinerea* were removed in the field whilst picking as this infection has been found to have a negative effect on wine colour (Keller et al. 1999).

Winemaking protocol was amended after the 2006 season due to the purchase of additional equipment for the micro winery. Only basic equipment was available for the 2006 season and more advanced equipment was purchased prior to the 2007 season. Protocol for the 2006 season is described in 3.2.3.1.1, and protocol for both the 2007 and 2008 seasons is described in 3.2.3.1.2.

#### 3.2.3.1.1 2006 SEASON

Treatments were harvested separately into sterilised (with citric acid and sulphur solution) picking buckets and then stored overnight in a cool room at 2 °C. The following day grapes were returned to ambient temperature before destemming and crushing treatments. Destemming was done by hand by rubbing bunches over the drum of an old small destemming machine over a long, shallow bucket to catch the must. Grapes were around 80 percent macerated by this method, and then further crushing was carried out by hand. Must was returned to re-sterilised buckets.

When the must had warmed to approximately 20 °C, yeast inoculation, sulphur and enzyme additions were carried out (described in section 3.2.3.1.3). Wines were fermented for five nights on skins, with twice daily manual punch downs, in an insulated room maintained at 20 °C. Treatments were rotated through the shelf space to ensure consistent fermentation temperatures in the warm fermentation room. The buckets were covered with lids.

After fermentation, the wine was pressed off skins by hand using a sample analysis bucket press (Plate 3-1) into 2 L or 3 L polyurethane (P.E.T.) containers. An initial headspace of around 3 cm was left per container, which was then compressed by squeezing the sides of the container so that there was no headspace and P.E.T. containers were capped. This allowed for the continuation of the fermentation until wines were dry as $CO_2$ accumulated in the headspace. This $CO_2$ was then released daily and headspace again compressed.

In 2006, wines underwent malolactic fermentation (CHR Hansen Viniflora bacteria) in the ambient temperature room and the progress of this fermentation was monitored using thin layer chromatography. This process was not carried out in following years due to the effect that malolactic fermentation can have on other wine properties such as sensory

characteristics (Sauvageot & Vivier 1997) and the desire to show only effects in wine due to treatments applied in the vineyard.

**Plate 3-1 Hand pressing of 2006 wines using a sample analysis press.**

#### 3.2.3.1.2 2007 AND 2008 SEASONS

In 2007 and 2008 seasons, the winemaking protocols were standard as described in section 3.2.3.1.3, but differed to 2006 methods in that a Baesso 80 kg crusher/destemmer unit (Australian Winemakers, Victoria, Australia) was used for initial processing and a submerged cap system (Figure 3-10) was used instead of manual punchdowns. The submerged cap system was achieved through the use of a solid plastic board that fitted snugly inside the 10 L buckets through which small holes had been drilled. Stainless steel wires were then bent in a zigzag to hold the header boards down in the lower third of the bucket to keep the cap submerged well below the liquid surface. Ferments were aerated after two days to ensure the health and progress of the fermentation.

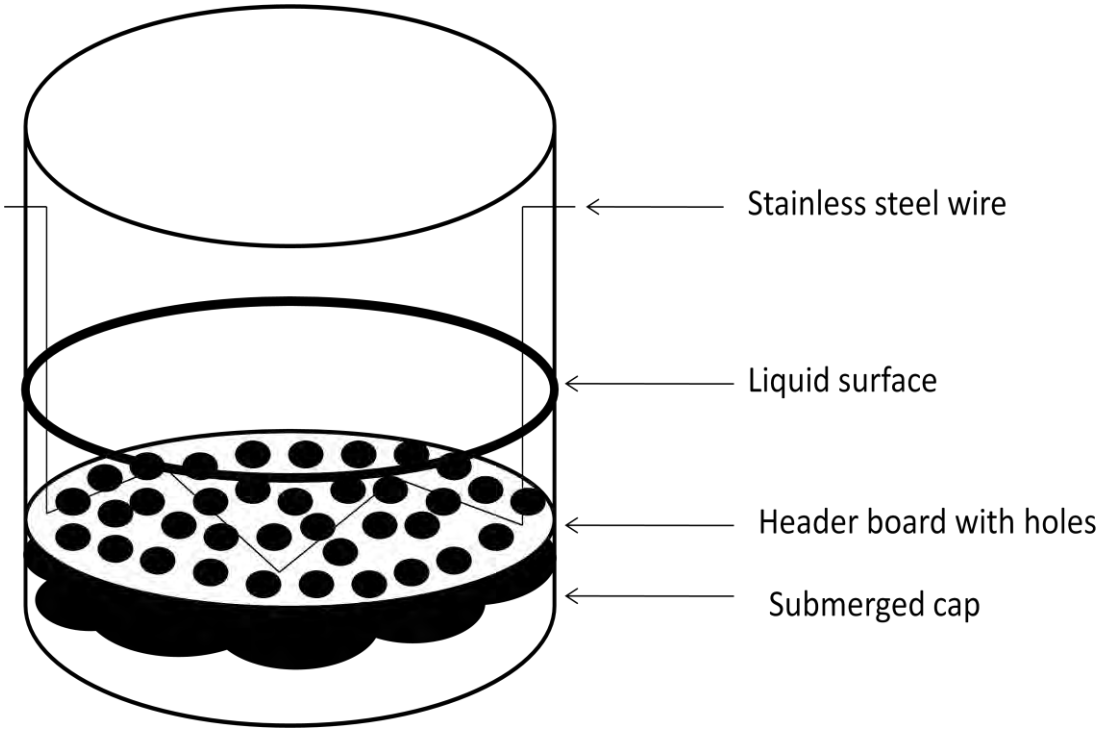


**Figure 3-10 Submerged cap system used in fermentation vessels in 2007 and 2008**

#### 3.2.3.1.3 STANDARD WINEMAKING PROTOCOL OVER ALL SEASONS

After destemming and crushing, must was returned to sterilised 10 L buckets and sulphur was added at a rate of 50 ppm $SO_2$ (added as potassium metabisulfite solution) to kill wild yeast and prevent oxidation. Sulphur solution was mixed well through the must. Lafase HE enzyme (Laffort, France) was added (1 mL of 3 percent stock solution per kg of crushed fruit) and mixed well through the must. Must was then moved to the warm room (28 °C) for 2-3 hours to allow the enzyme to activate, to allow the sulphur to become bound and to warm the must to a desirable temperature for yeast addition (25-28 °C).

Yeast RC 212 (Lallemand, Canada) was rehydrated according to manufacturer's instructions and added at a rate of 300 ppm to the warmed must. After half an hour or so, the must was mixed to distribute the yeast. Buckets were loosely fitted with lids. Fermentation temperature was maintained between 25-28 °C for the duration of the ferment and ferments were monitored daily by either hydrometer or Anton Parr portable density meter DMA 35N (Anton Parr GmbH, Graz, Germany).

After fermentation for five nights on skins, ferments were below 2 °Be and wines were then pressed off the skins using a 20 L Idro water bag press (Australian Winemakers, Victoria, Australia). During pressing, care was taken to endeavour to keep the wine under a cover of dry ice to prevent oxidation. Wines were pressed into 3 L P.E.T. bottles (Caled Containers, Tasmania), leaving a small headspace that was compressed to allow space for $CO_2$ to accumulate during the completion of fermentation. $CO_2$ was then released daily and the headspace compressed again. This was repeated until sugar levels in the wines were below 0.25 g/L as determined by Clinitest® Reagent tablets. When fermentation was finished, 40 ppm $SO_2$ was added and then wine was left to settle for 5 days. After 5 days, wines were racked off lees by pressure transfer under the cover of nitrogen gas and free $SO_2$ analysed (Iland 2000) to reach a level between 30 and 40 ppm. Once desirable free $SO_2$ levels were reached and maintained, wines were then bottled under the cover of nitrogen gas. Wines were passed through two membrane cartridge filters in series (Tenco, Italy) of pore size 1 µm and 0.25 µm.

#### *3.2.3.2 WINE ANALYSES*

Wines were analysed at bottling time, approximately three months after the completion of fermentation. Wines analysed at this time are referred to as new wines and the age of wines is taken from this point for the 12 month and 24 month old wines. Wines from the 2006 season were not analysed until they had aged for 12 and 24 months and were then analysed according to the methods of Iland (2000) based on the original measures of Somers and Evans (1974) and Somers and Evans (1977). Absorbance readings were taken at 420 nm and 520 nm, enabling calculation of $SO_2$ corrected wine colour density ($SO_2$ WCD), chemical age 1, hue and $SO_2$ resistant pigment.

The use of $SO_2$ WCD in the current study as opposed to the original wine colour density measure is due to $SO_2$ WCD being a more accurate reflection of wine colour density. This modified wine colour density takes into account that anthocyanins can be bleached from winemaking sulphur additions, and the addition of acetaldehyde reverts the bleached anthocyanins back to the coloured flavylium form due to acetaldehyde preferentially binding to the anthocyanin molecules over $SO_2$ (Mercurio et al. 2007, Somers & Evans 1977).

Research winemaking practices (3.2.3.1) used blanket $SO_2$ additions, so use of $SO_2$ WCD allowed for varying free $SO_2$ levels. Chemical age 2 was not measured in 2006.

New wines from 2007 and 2008 were analysed by the modified Somers method (Mercurio et al. 2007) which in addition to the original Somers method, measures the degree of ionisation of anthocyanins, total anthocyanins, $SO_2$ resistant pigment and total phenolics. Total pigment was also measured (Iland 2000). The modified Somers method had the added benefit of analysing the wines at standard pH and alcohol thus reducing the effect that pH and alcohol can have on wine colour (Mercurio et al. 2007). Wine tannin measurements were taken for new 2008 wines using a spectrophotometric method developed by Dr R Dambergs (pers. comm.) of the Australian Wine Research Institute (AWRI) using a range of wavelengths between 200 nm and 520 nm. Total pigment was also calculated from this sample (R. Dambergs pers. comm.).

**$SO_2$ corrected wine colour density (AU):**

$(A_{420acetaldehyde} + A_{520acetaldehyde}) \times 10$

**Chemical age 1 (no units):**

$A_{520sulphite}/A_{520acetaladehyde}$

**Chemical age 2 (no units):**

$A_{520sulphite}/(5 \times A_{520HCl})$

**Hue (no units):**

$A_{420buffer1}/A_{520buffer1}$

**Total anthocyanins (mg/L):**

$20 \times [(50 \times A_{520HCl}) - 1.6667 \times (10 \times A_{520sulphite})]$

**$SO_2$ resistant pigment (AU):**

$A_{520sulphite} \times 10$

**Degree of ionisation of anthocyanins (%):**

$$\left\{ \frac{(10 \times A_{520buffer1}) - (10 \times A_{520sulphite})}{(50 \times A_{520HCl}) - [1.6667 \times (10 \times A_{520sulphite})]} \right\} \times 100$$

**Total phenolics (AU):**

$(A_{280HCl} \times 50) - 4$

**Total pigment (AU):**

$51 * A_{520HCl}$

## 3.2.4 STATISTICAL ANALYSES

### *3.2.4.1 ANALYSIS OF VARIANCE*

Data analysis was carried out using general analysis of variance (ANOVA) or the 'Factorial plus Added Control' model in GenStat Release 12.1.0.3338 (VSN International Ltd., U.K.) and it is detailed in each experimental chapter which method was used. Fisher's Protected Least Significant Difference (LSD) was calculated at 5 percent probability (F pr.) level.

### *3.2.4.2 PRINCIPAL COMPONENT ANALYSIS*

Principal component analysis (PCA) involves a mathematical procedure that transforms a number of possibly correlated variables into a smaller number of uncorrelated latent variables called principal components (PCs). The data is reduced to a new set of values that best describe the difference between the samples. The first PC accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. The procedure was performed using The Unscrambler, version 9.8 (Camo, Norway). To account for scaling differences among the variables, data was first standardised by dividing values by the standard deviation for the variable. Cross-validation was used during the calculations (typically 20 groups with 5 samples per group) and data was centred. The key parameters derived by the calculations were the principal component scores, and the loadings to derive those scores. In a plot of PC scores, clustering of related samples can be observed and relationships between variables can also be observed.

When compared with sample information, the score plots can identify possible variation in the data related to experimental treatments.

Wines from the 2006 season were not analysed until they had aged for 12 months, hence two sets of data were analysed by PCA. The first data set included yield, yield component, fruit composition and seasonal weather condition data of variables measured in each of the three experimental seasons (2006, 2007 and 2008). To include wine composition data, new wine composition data was added to the previous data and a second set of data from the 2007 and 2008 seasons was analysed by PCA. The HPLC data had a high degree of colinearity, so to aid clarity of presentation was not included in the PCA data.

# CHAPTER 4: THE EFFECT OF WINTER PRUNING ON PINOT NOIR FRUIT AND WINE COMPOSITION

## 4.1 INTRODUCTION

Winter pruning to a set node number is essential in cool climates to limit yield to a level that can be sufficiently ripened as is the case for Pinot Noir in Tasmania. A primary aim when choosing the pruning level, is to achieve balance between vegetative and fruit growth whilst simultaneously providing appropriate fruit composition for winemaking (Jackson & Lombard 1993, Tassie & Freeman 1988). This balance needs to be sustainable across a number of years, measurable by continued high yields, maintenance of vine size and maintenance of quality wood (Kimball & Shaulis 1958).

Pruning level can have variable effects on both vegetative and fruit growth, and other factors such as seasonal weather conditions are also important. Further, there is compensation amongst growth and yield components, primarily budburst and fruitset, so the relationship of yield components to node number is not a simple linear function (Jackson et al. 1984). Increasing pruning severity can increase shoot number per node, increase bunch weight (Jackson et al. 1984) and increase fruitfulness (Byrne & Howell 1978). Compensatory lateral growth resulting from heavier pruning can be very important in affecting the total amount of vegetative growth.

### 4.1.1 THE IMPACT OF WINTER PRUNING ON YIELD AND YIELD COMPONENTS

Many studies across a range of varieties have shown the sensitivity of yield and yield components in response to variation of node number (Jackson & Lombard 1993, Jackson et al. 1984, Tassie & Freeman 1988, Zamboni et al. 1996). Early studies of vine balance have shown an increase in yield per vine with an increase in node number retained at pruning (Kimball & Shaulis 1958, Shaulis & Oberle 1948, Tompkins & Shaulis 1955), however more recent studies have refined this response showing that increasing node number generally increases yield until a plateau is reached, after which yield increases at a much slower rate (Bindon et al. 2008, Jackson & Lombard 1993, Jackson & Steans 1983-4, Jackson et al. 1984, Tassie & Freeman 1988).

Of all yield components, percent budburst is the most sensitive as it has been shown that yield stabilisation at high node numbers is due to a reduction in budburst (Jackson & Steans 1983-4). Shoot number per node can also be decreased with decreasing pruning severity (Jackson et al. 1984). Fruitfulness is often decreased with decreasing pruning severity (Byrne & Howell 1978), however Tomkins and Shaulis (1955) found that an increase in yield with increasing node number more than offset the resulting decrease in fruitfulness due to increased node number.

Yield component compensation can result in pruning level alone being an inadequate management tool for yield manipulation as berry number can change at fruitset and berry size can change during the season (Dry et al. 2004). Dunn et al. (2004) found that more severe pruning increased berry size in Chardonnay and Cabernet Sauvignon and warned that for cool-climate cane pruned vines, responses were less consistent than in warmer climates. Bindon et al. (2008) found only a weak relationship between berry size and secondary metabolite concentration in Shiraz utilising a wide range of berry sizes as a result of winter pruning treatments.

### 4.1.2 THE IMPACT OF WINTER PRUNING ON FRUIT COMPOSITION

The change in yield that is seen as a response to alteration of winter pruning levels is also reflected in fruit composition. Early studies found increasing yield delayed maturity by lowering sugar (total soluble solids [TSS]) and titratable acidity (TA) in berries at harvest (Winkler 1970). A delay in maturity can become very important in cool and marginal climates where harvest delay could result in problems with autumn rainfall or frost.

When node number was increased 3.5-fold in New Zealand's cool climate, there was little effect on basic fruit composition (TSS, pH and TA) across four cultivars (Chardonnay, Sauvignon Blanc, Gewurztraminer and Cabernet Sauvignon) (Jackson & Steans 1983-4, Jackson et al. 1984). Trials on Muller Thurgau indicated an upper threshold of 72 nodes per vine, beyond which grape maturity was delayed and TSS and pH decreased, and TA increased (Jackson & Steans 1983-4). This upper threshold concept was further explored by Bravdo et al. (1985) and Bravdo et al. (1984) in warmer climates, with Cabernet Sauvignon and

Carignane, respectively in Israel. They found that beyond this upper threshold a decrease in fruit and wine qualities were observed. In a warmer climate (mean January temperature 21-23 °C) Bindon et al. (2008) found the plateau to be at around 120 nodes per vine for Shiraz after which TSS ripeness was delayed and pH, TA, anthocyanins and phenolics were not significantly affected.

Heazlewood et al. (2006) found that a significant delay in Pinot Noir maturity was not observed when increasing node number per vine up to 40 nodes per vine in the cool climate region of Tasmania. No significant differences in TSS was observed between seasons or treatments in this study, however, there was a significant decrease in grape pH at higher node numbers. This study did not carry viticultural treatments through to winemaking. Early studies by Shaulis and Robinson (1953) showed the rate of maturity of Concord and Fredonia was more affected by seasonal variation than by pruning severity treatments.

### 4.1.3 THE IMPACT OF WINTER PRUNING ON WINE COMPOSITION

High yields have been shown to delay maturity and to decrease wine quality for some red varieties (Chapman et al. 2004, Jackson & Schuster 1987, McCarthy et al. 1987, Winkler 1954, Winkler 1970). Bravdo et al. (1984) and Bravdo et al. (1985) found that the wine colour density of Carignane and Cabernet Sauvignon decreased when yields were promoted beyond the upper yield limit for each variety. This indicates a variety and region specific yield threshold or plateau beyond which wine quality declines. To the best of the author's knowledge, few trials have taken Pinot Noir fruit from winter pruning level treatments through to wine composition assessment.

### 4.1.4 EXPERIMENTAL APPROACH

The current study investigated the fruit and wine composition responses of cool climate Pinot Noir clone 114 in response to variation of node number retained at winter pruning over three seasons. Pinot Noir wines often do not develop stable wine colour and this trial also investigated if colour stability could be influenced in the vineyard by pruning level.

## 4.2 MATERIALS AND METHODS

General materials and methods are covered in Chapter 3. Node number per vine was manipulated at commercial pruning time in 2005 (2006 season), 2006 (2007 season) and 2007 (2008 season). Pruning treatment were achieved by pruning to 10 nodes per cane, thus for the 10 nodes per vine treatment, 1 cane was laid down, for the 20 nodes per vine treatment, 2 canes were laid down, for the 30 nodes per vine treatment, 3 canes were laid down and for the 40 nodes per vine treatments, 4 canes were laid down. The same vines received the same treatment in consecutive seasons. Vines were commercially managed for the duration of the project on a Scott Henry trellis system (Freeman et al. 1988, Smart & Robinson 1991, Smart 1988). Fruit from all treatments was harvested on the same day, being 4$^{th}$ April 2006, 3$^{rd}$ April 2007 and 28$^{th}$ March 2008 (coinciding with commercial harvest).

### 4.2.1 EXPERIMENTAL DESIGN

The 2006, 2007 and 2008 season's statistical design was a randomised complete block design with four treatments and four replicates. Each replicate was a block in separate partial vineyard rows. Treatments were 10, 20, 30 or 40 nodes per vine, resulting in (3.3, 6.7, 10.0 or 13.3 nodes per metre canopy respectively on the Scott Henry trellis or 11 100, 20 000, 28 900 or 37 800 m$^2$ per hectare respectively of exposed canopy surface area. These treatments were designed to achieve node numbers either side of the average Tasmanian commercial pruning level of approximately 30 nodes per vine, 10.0 nodes per metre canopy or 28 900 m$^2$ per hectare of exposed canopy surface area when trained to a Scott Henry trellis system. A plot was four adjacent vines with the outer two vines on each side as buffers. For each plot all results were taken as the mean per vine calculated from measurements on the central two vines.

**Plate 4-1** **10 nodes per vine pruning level (10 nodes laid down on one cane).**

**Plate 4-2** **20 nodes per vine pruning level (20 nodes laid down on two canes, 10 nodes per cane).**

### 4.2.2 STATISTICAL ANALYSES

Data was analysed by analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (LSD) using GenStat Release 12.1.0.3338 (VSN International Ltd., U.K.) The LSD was calculated at 5 percent probability (F pr.) level. Data was also analysed by principal component analysis (PCA) as described in Chapter 3 using The Unscrambler, version 9.8 (Camo, Norway). The first data set from the 2006, 2007 and 2008 season included 16 yield, yield component, fruit composition and seasonal weather condition variables. The second data set from the 2007 and 2008 seasons included 24 yield, yield component, fruit composition, new wine composition and seasonal weather condition variables.

## 4.3 RESULTS

### 4.3.1 YIELD AND YIELD COMPONENTS

Node number treatments did not significantly affect bunch weight (Table 4-1). Node number and season factors significantly interacted resulting in a lack of node number treatment effect within the 2006 and 2007 seasons (Figure 4-1). In the 2008 season, 10 nodes per vine pruning level resulted in the lowest bunch weight (81.6 g) and 30 nodes per vine the highest (112.3 g).

**Table 4-1 Probability for fruitfulness, harvest bunch weight and yield for the 2006, 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Bunch weight (g) | Fruitfulness (bunches/node) | Yield (kg/vine) |
|---|---|---|---|
| Node number | 0.281 | <.001 | <.001 |
| Season | 0.042 | <.001 | <.001 |
| Node number.Season | 0.046 | <.001 | 0.066 |

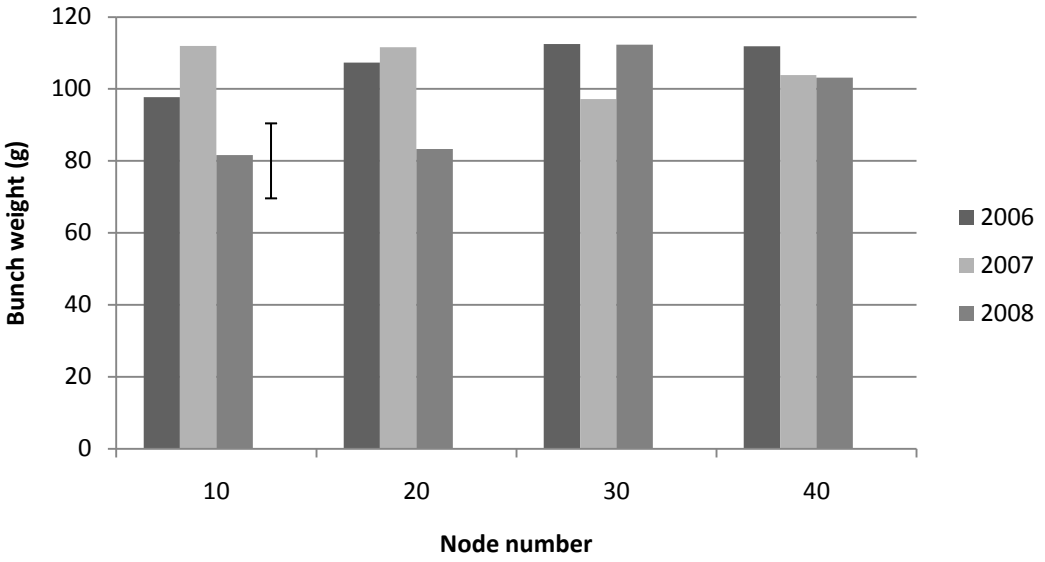


**Figure 4-1 The interaction effect of node number and season factors on bunch weight for 2006, 2007 and 2008 seasons. Data points are means of four replicates. The bar represents the least significant difference between data points.**

Fruitfulness, measured as bunches per node in line with manipulation of node number treatments, was significantly influenced by the interaction between node number and season treatments (Figure 4-2). In the 2006 season, 10 nodes per vine pruning level had higher fruitfulness (2.1 bunches/node) than other pruning levels. In the 2007 season, 10 nodes per vine was highest (1.6 bunches/node) and 30 nodes per vine was lowest (1.1 bunches/node). In the 2008 season, pruning level treatments were not significantly different from one another.

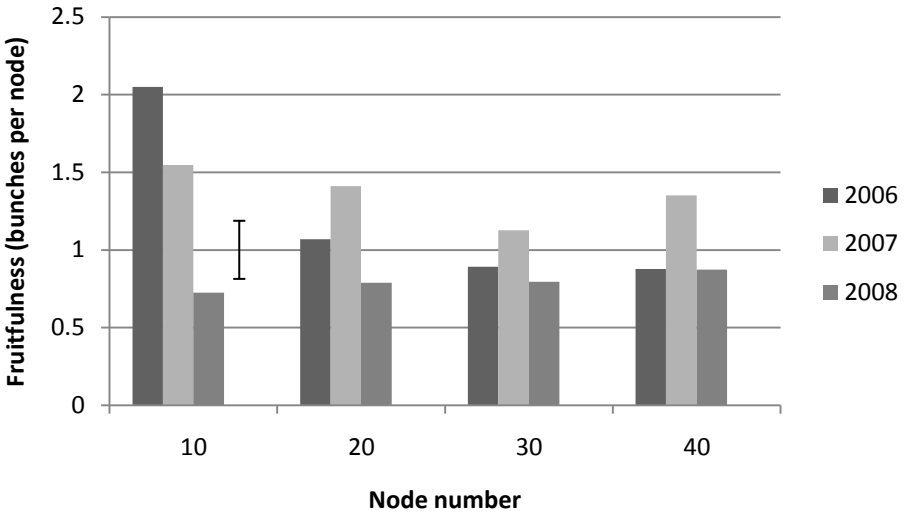


**Figure 4-2 The interaction effect of node number and season on fruitfulness for 2006, 2007 and 2008 seasons. Data points are means of four replicates. The bar represents the least significant difference between data points.**

Increasing node number per vine increased yield per vine (Figure 4-3). Yield for 10 nodes per vine (2.27 kg/vine or 6.7 t/ha) and 20 nodes per vine (3.03 kg/vine or 9.0 t/ha) were not significantly different from one another, but 30 nodes per vine (4.57 kg/vine or 13.5 t/ha) and 40 nodes per vine (5.68 kg/vine or 16.8 t/ha) were significantly different from all other treatments and each other. Average yields were significantly higher in the 2006 season (6.11 kg/vine or 18.1 t/ha) than 2007 (2.74 kg/vine or 8.1 t/ha) and 2008 (2.80 kg/vine or 8.3 t/ha).

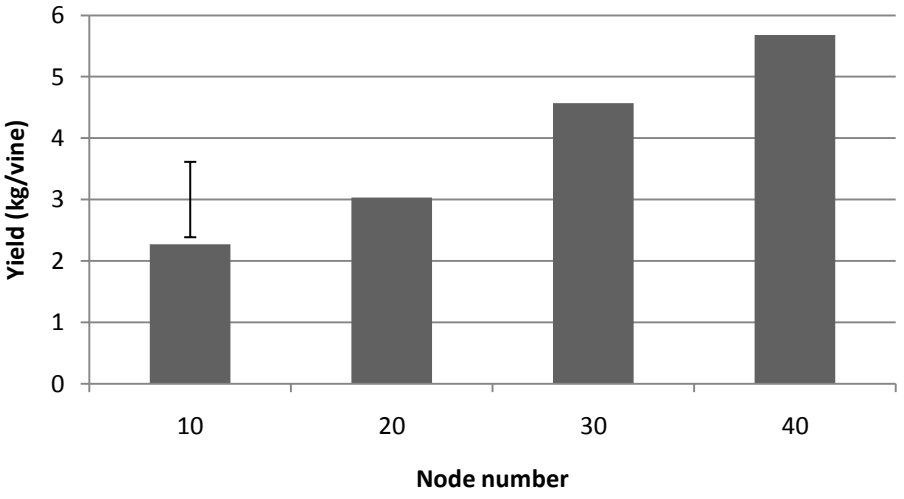


**Figure 4-3 The effect of node number on yield for 2006, 2007 and 2008 seasons. Data points are means of four replicates and three seasons. The bar represents the least significant difference between data points.**

The 2006 season had lower cane weight (65.4 g) than either 2007 (77.6 g) or 2008 (88.7 g) (Table 4-2). Cane weight decreased as node number increased with 10 nodes per vine (106.8 g) and 20 nodes per vine (94.2 g) significantly heavier than 30 nodes per vine (63.0 g) or 40 nodes per vine (44.9 g) (Figure 4-4).

**Table 4-2 Probability for cane weight, yield to pruning weight ratio and berry weight for the 2006, 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Cane weight (g) | Yield to pruning weight ratio | Berry weight (g) |
|---|---|---|---|
| Node number | <.001 | 0.007 | 0.002 |
| Season | 0.002 | <.001 | 0.183 |
| Node number.Season | 0.293 | 0.982 | 0.885 |

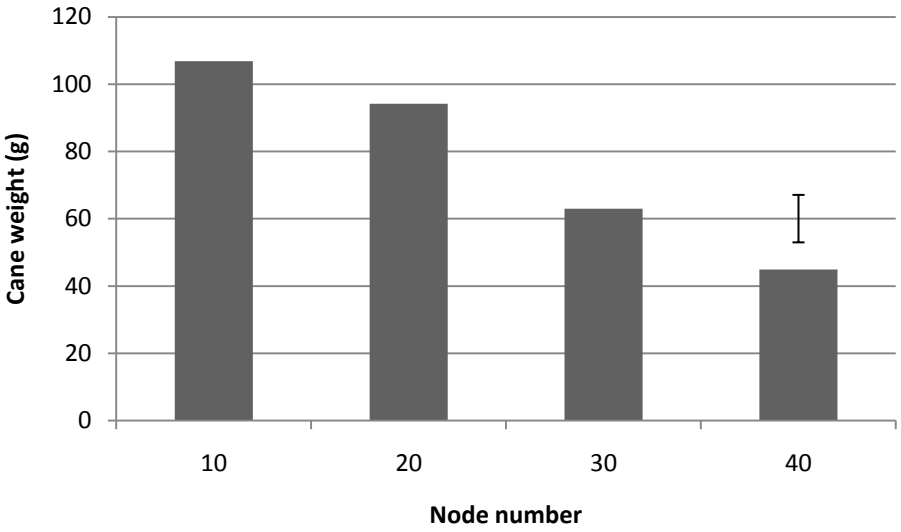


**Figure 4-4 The interaction effect of node number on cane weight for 2006, 2007 and 2008 seasons. Data points are means of four replicates and three seasons. The bar represents the least significant difference between data points.**

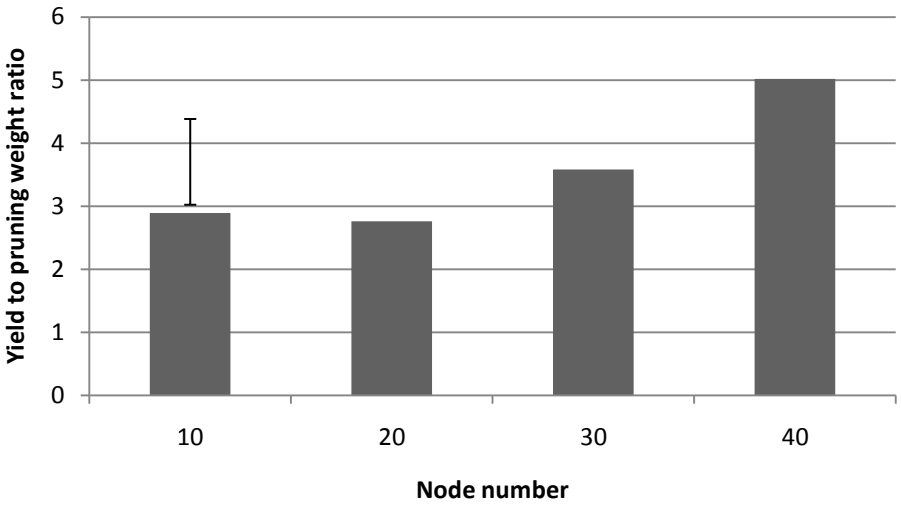


**Figure 4-5 The interaction effect of node number on yield to pruning weight ratio for 2006, 2007 and 2008 seasons. Data points are means of four replicates and three seasons. The bar represents the least significant difference between data points.**

Yield to pruning weight ratio (Y:P) was higher in the 2006 season (6.08) than 2007 (2.67) and 2008 (1.94) seasons. As node number per vine increased, so too did Y:P (Figure 4-5). Berry weight was significantly reduced as node number increased in the 2007 and 2008 seasons (Table 4-2). The 40 nodes per vine treatment was lowest (1.37 g) with the other three treatments not significantly different from one another (Figure 4-6).

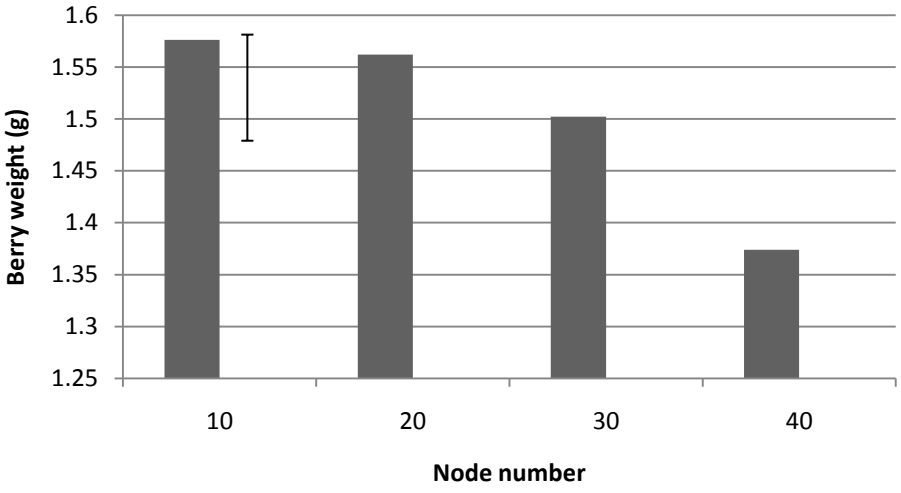


**Figure 4-6 The effect of node number on berry weight for 2007 and 2008 seasons. Data points are means of four replicates and two seasons. The bar represents the least significant difference between data points.**

The incidence of *Botrytis cinerea* was not significantly affected by node number treatments in the 2008 season, the only season in which it was measured (Table 4-3).

**Table 4-3 Probability for incidence of *Botrytis cinerea* for the 2008 season. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Bunches with Botrytis (%) |
|---|---|
| Node number | 0.390 |

### 4.3.2 FRUIT COMPOSITION

Node number treatments did not significantly affect pH of grapes, but mean pH from the 2006 season (3.54) was higher than 2007 (3.30) and 2008 (3.30) (Table 4-4). Total soluble solids (TSS) were significantly influenced by the interaction between node number treatments and season (Figure 4-7). Within the 2006 season TSS decreased as node number increased, within the 2007 season TSS increased up to 30 nodes per vine and then decreased to 40 nodes per vine and in the 2008 season there was no significant effect of node number, but TSS tended to decrease as node number increased.

**Table 4-4 Probability of total soluble solids (TSS), pH and titratable acidity (TA) of grapes for the 2006, 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | TSS (°Be) | pH (no units) | TA (g/L) |
|---|---|---|---|
| Node number | 0.052 | 0.632 | <.001 |
| Season | <.001 | <.001 | <.001 |
| Node number.Season | 0.006 | 0.448 | <.001 |

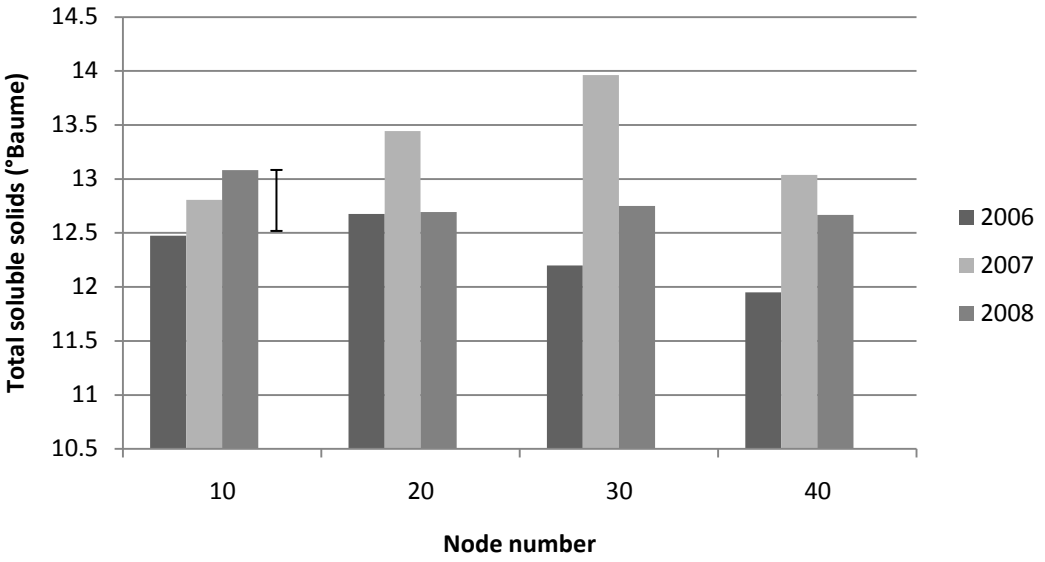


**Figure 4-7 The interaction effect of node number and season on total soluble solids for 2006, 2007 and 2008 seasons. Data points are means of four replicates. The bar represents the least significant difference between data points.**

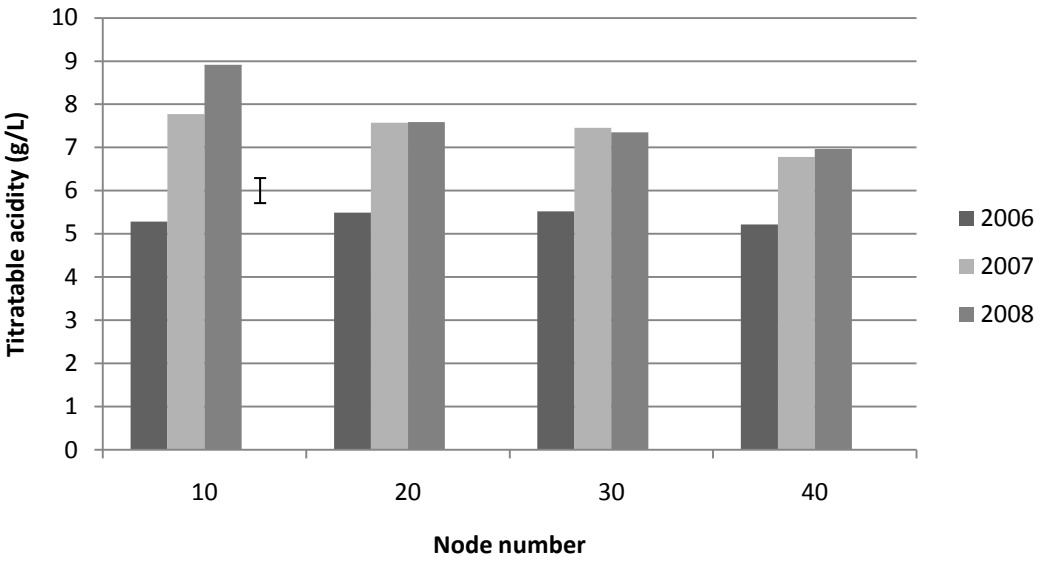


**Figure 4-8 The interaction effect of node number and season on titratable acidity for 2006, 2007 and 2008 seasons. Data points are means of four replicates. The bar represents the least significant difference between data points.**

Titratable acidity (TA) was significantly influenced by the interaction between node number treatments and season (Figure 4-8). Within the 2006 season there were no significant

differences between treatments, in the 2007 season 40 nodes per vine (6.79 g/L) was significantly lower than other node number treatments. In the 2008 season, TA decreased as node number increased, with 10 nodes per vine (8.91 g/L) significantly higher than all other treatments.

Grape total anthocyanins, analysed by spectroscopy, were significantly different between season with 2006 the lowest (0.44 mg/g), 2007 the highest (0.70 mg/g) and 2008 intermediate (0.55 mg/g). Grape total phenolics were only measured in 2007 and 2008 seasons and the 2007 season was significantly higher (1.63 AU) than 2008 (0.99 AU).

**Table 4-5 Probability of grape total anthocyanins analysed by spectroscopy for the 2006, 2007 and 2008 seasons and grape total phenolics analysed by spectroscopy for the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Grape total anthocyanins by spectroscopy (mg/g) | Grape total phenolics by spectroscopy (AU) |
|---|---|---|
| Node number | 0.069 | 0.816 |
| Season | <.001 | <.001 |
| Node number.Season | 0.79 | 0.228 |

Grape total anthocyanins, analysed by HPLC, showed a significant difference between seasons and were higher in 2007 (1.25 mg/g) than 2008 (1.00 mg/g) (Table 4-6). Cyanidin-3-glucoside (c3g) was also higher in 2007 (0.032 mg/g) than 2008 (0.021 mg/g), as was delphinidin-3-glucoside in 2007 (0.048 mg/g) compared to 2008 (0.024 mg/g). Node number also had a significant effect on cyanidin-3-glucoside with varying concentrations as node number increased (Figure 4-9).

**Table 4-6 Probability of grape total anthocyanins, cyanidin-3-glucoside and delphinidin-3-glucoside at harvest analysed by HPLC for the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Grape total anthocyanins by HPLC (mg/g) | Cyanidin-3-glucoside (mg/g) | Delphinidin-3-glucoside (mg/g) |
|---|---|---|---|
| Node number | 0.16 | 0.044 | 0.315 |
| Season | <.001 | <.001 | <.001 |
| Node number.Season | 0.396 | 0.107 | 0.19 |

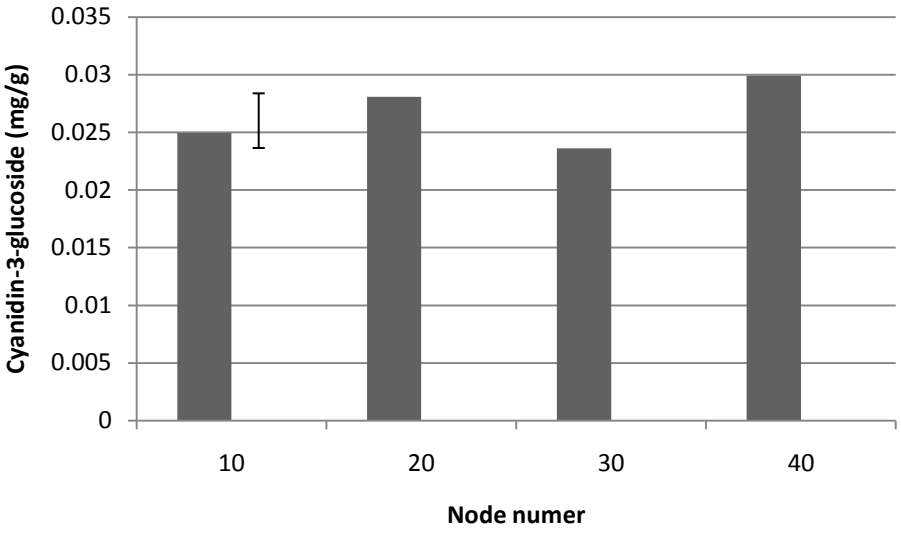


**Figure 4-9 The interaction effect of node number on cyanidin-3-glucoside at harvest analysed by HPLC for 2007 and 2008 seasons. Data points are means of four replicates and two seasons. The bar represents the least significant difference between data points.**

Malvidin-3-glucoside was significantly higher in the 2007 season (0.70 mg/g) than the 2008 season (0.60 mg/g) (Table 4-7) as was peonidin-3-glucoside (0.40 mg/g and 0.30 mg/g respectively) and petunidin-3-glucoside (0.08 mg/g and 0.05 mg/g respectively). Peonidin-3-glucoside varied as node number increased, but appeared to follow a similar trend to cyanidin-3-glucoside, with 20 and 40 nodes per vine higher than 10 and 30 nodes per vine (Figure 4-10).

**Table 4-7 Probability of malvidin-3-glucoside, peonidin-3-glucoside and petunidin-3-glucoside at harvest analysed by HPLC for the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Malvidin-3-glucoside (mg/g) | Peonidin-3-glucoside (mg/g) | Petunidin-3-glucoside (mg/g) |
|---|---|---|---|
| Node number | 0.369 | 0.005 | 0.222 |
| Season | 0.005 | <.001 | <.001 |
| Node number.Season | 0.56 | 0.503 | 0.205 |

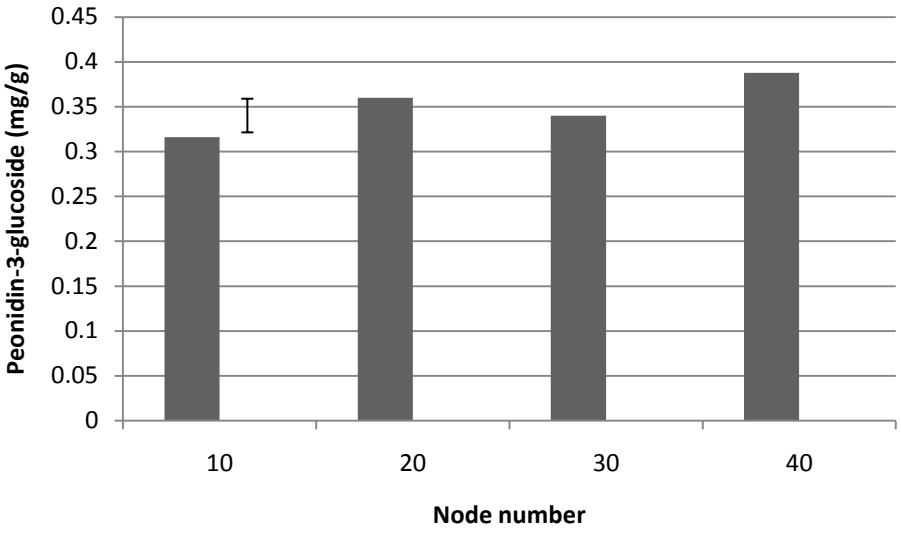


**Figure 4-10 The interaction effect of node number on peonidin-3-glucoside at harvest analysed by HPLC for 2007 and 2008 seasons. Data points are means of four replicates and two seasons. The bar represents the least significant difference between data points.**

## 4.3.3 WINE COMPOSITION

### *4.3.3.1 NEW WINES*

Node number did not significantly affect the $SO_2$ corrected colour density ($SO_2$ WCD), chemical age 1 or chemical age 2 of new wines from the 2007 and 2008 seasons (Table 4-8). Differences between seasons were observed for $SO_2$ WCD which was higher in 2007 (4.78 AU) than 2008 (3.62 AU) and chemical age 1 which was lower in 2007 (0.19) than 2008 (0.21).

**Table 4-8 Probability for $SO_2$ corrected wine colour density, chemical age 1 and chemical age 2 of new wine from the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | $SO_2$ corrected colour density (AU) | Chemical age 1 (no units) | Chemical age 2 (no units) |
|---|---|---|---|
| Node number | 0.159 | 0.977 | 0.909 |
| Season | <.001 | 0.003 | 0.918 |
| Node number.Season | 0.087 | 0.96 | 0.63 |

Node number treatments did not affect hue, total anthocyanins or total phenolics of new 2007 and 2008 wines (Table 4-10). The 2007 season resulted in significantly higher

anthocyanins and phenolics (553 mg/L and 72.1 mg/L respectively) than 2008 (445 mg/L and 45.2 mg/L respectively).

**Table 4-9 Probability for hue, total anthocyanins and phenolics of new wine from the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Hue (no units) | Total anthocyanins (mg/L) | Total phenolics (AU) |
|---|---|---|---|
| Node number | 0.3 | 0.793 | 0.271 |
| Season | 0.096 | 0.006 | <.001 |
| Node number.Season | 0.5 | 0.359 | 0.771 |

Node number treatments did not affect the degree of ionisation of anthocyanins, total pigment or concentration of $SO_2$ resistant pigment in new 2007 and 2008 wines (Table 4-10). New wines from 2007 were significantly higher in ionisation of anthocyanins and $SO_2$ resistant pigment (7.6 percent and 0.57 AU respectively) than wines from the 2008 season (3.9 percent and 0.47 AU respectively). Dambergs tannin of new wines from the 2008 season did not show any significant node number treatment effects (Table 4-12).

**Table 4-10 Probability for degree of ionisation of anthocyanins, total pigment and $SO_2$ resistant pigment of new wine from the 2007 and 2008 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Degree of ionisation of anthocyanins (%) | Total pigment (AU) | $SO_2$ resistant pigment (AU) |
|---|---|---|---|
| Node number | 0.948 | 0.79 | 0.19 |
| Season | <.001 | 0.213 | <.001 |
| Node number.Season | 0.654 | 0.52 | 0.182 |

**Table 4-11 Probability for Dambergs tannin of new wine from the 2008 season. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Dambergs tannin (g/L) |
|---|---|
| Node number | 0.920 |

#### *4.3.3.2 12 MONTH OLD WINES*

Node number treatments did not significantly affect $SO_2$ WCD, chemical age 1, hue or $SO_2$ resistant pigment of 12 month old wines from the 2006 and 2007 seasons (Table 4-12). Wines from the 2006 season were lower in $SO_2$ WCD and $SO_2$ resistant pigment (3.32 AU and 0.65 AU respectively) than the wines from the 2007 season (5.39 AU and 1.10 AU respectively). Hue of 12 month old wines from the 2006 season was significantly higher (0.89) than wines from the 2007 season (0.64). Increasing node number decreased hue with 10 nodes per vine (0.79) highest and 30 nodes per vine the lowest (0.74) (Figure 4-11).

**Table 4-12 Probability for $SO_2$ corrected wine colour density, chemical age 1 and hue of 12 month old wine from the 2006 and 2007 seasons. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | $SO_2$ corrected colour density (AU) | Chemical age 1 (no units) | Hue (no units) | $SO_2$ resistant pigment (AU) |
|---|---|---|---|---|
| Node number | 0.636 | 0.319 | 0.002 | 0.358 |
| Season | <.001 | 0.051 | <.001 | <.001 |
| Node number.Season | 0.707 | 0.39 | 0.051 | 0.531 |

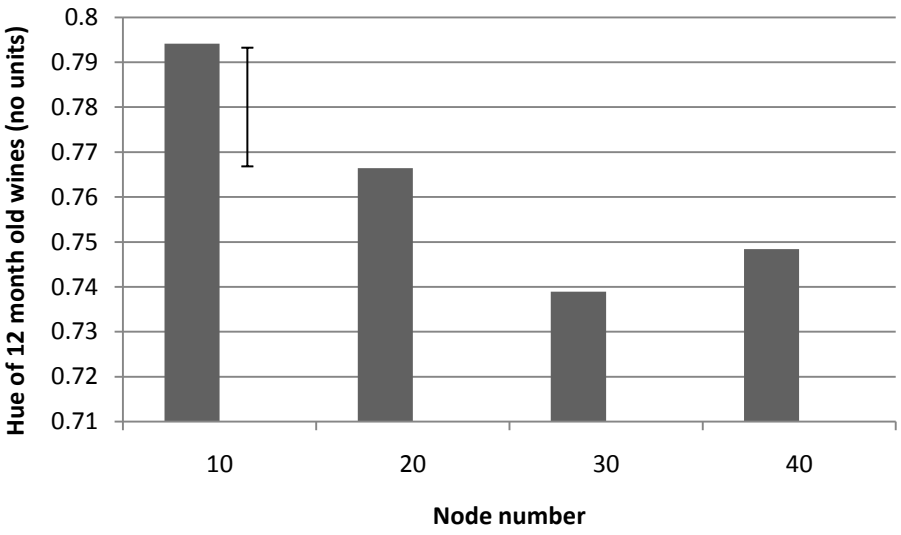


**Figure 4-11 The interaction effect of node number on hue of 12 month old wines from the 2006 and 2007 seasons. Data points are means of four replicates and two seasons. The bar represents the least significant difference between data points.**

Additional wine composition variables measured on 12 month old 2007 wines of chemical age 2, total anthocyanins, total phenolics, ionisation of anthocyanins, total pigment and Dambergs

tannin did not show significant effects of node number treatments (Table 4-13 and Table 4-14).

**Table 4-13 Probability for chemical age 2, total anthocyanins and phenolics of 12 month old wine from the 2007 season. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Chemical age 2 (no units) | Total anthocyanins (mg/L) | Total phenolics (AU) |
|---|---|---|---|
| Node number | 0.363 | 0.487 | 0.756 |

**Table 4-14 Probability for degree of ionisation of anthocyanins, total pigment and Dambergs tannin of 12 month old wine from the 2007 season. Data shown are main treatment effects and treatment interaction effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| Source of variation | Degree of ionisation of anthocyanins (%) | Total pigment (AU) | Dambergs tannin (g/L) |
|---|---|---|---|
| Node number | 0.291 | 0.452 | 0.943 |

### 4.3.4 PRINCIPAL COMPONENT ANALYSIS (PCA)

Principal component 1 (PC 1) accounted for 50 percent of variability in the yield, yield components, fruit composition and seasonal weather condition data from the 2006, 2007 and 2008 seasons (Figure 4-12). Principal component 2 (PC 2) accounted for 19 percent of variability. PC 1 was positively loaded for titratable acidity (TA), growing season degree days (DD Sep-May), total soluble solids (TSS), grape anthocyanins, mean January temperature (MJT), mean February temperature (MFT) and mean cane weight. PC 1 was negatively loaded for rainfall, both for the growing season (Sep-May) and for annual rainfall from July-June, degree days up to the end of flowering (DD Sep-Dec), yield, yield to pruning weight ratio (Y:P) and pH. PC 2 was positively loaded for MFT, fruitfulness, bunch weight, grape anthocyanins, Y:P and yield. Negatively loaded on PC 2 were MJT, DD Sep-Dec, cane weight, TA, and both annual and growing season rainfall.

Separation of data points according to season was clearly shown in Figure 4-13, with PC 1 separating 2006 from the 2007 and 2008 seasons. PC 2 separated the 2007 and 2008 seasons, with the 2006 season relatively neutral for this component. Data points labelled according to node number treatments separated the two extremes of 10 and 40 nodes per vine from each

other primarily along PC 1, but also on PC 2. Treatments of 20 and 30 nodes per vine clustered in between the two extremes (Figure 4-14).

Analysis of 2007 and 2008 season data including new wine composition data resulted in PC 1 explaining 49 percent of variability in the data and PC 2 15 percent (Figure 4-15). Analysis of these two seasons changed the position of loadings as there was not the influence of the higher than average rainfall season of 2006 and most variables were positively loaded on PC 1. PC 1 was positively loaded for all new wine composition variables excepting hue, MFT, annual rainfall, DD Sep-May, fruitfulness, grape anthocyanins and phenolics, TSS, Y:P, bunch and berry weight. PC 1 was negatively loaded for MJT, DD Sep-Dec, growing season rainfall, hue, cane weight and TA. Positively loaded on PC 2 were yield, Y:P and bunch weight and negatively loaded on PC 2 were berry weight, TA, cane weight, new wine total pigment and TSS.

The 2007 and 2008 seasons separated clearly on PC 1 with 2007 positively loaded on this component and 2008 negatively (Figure 4-16). Within the two seasonal clusters, the two extreme treatments of 10 and 40 nodes per vine separated on PC 2, more clearly within the 2008 seasonal cluster than the 2007 season (Figure 4-17). In the 2007 seasonal cluster, 10 and 40 nodes per vine treatments separated along PC 1. The 20 and 30 nodes per vine treatments clustered in between the two more extreme treatments.

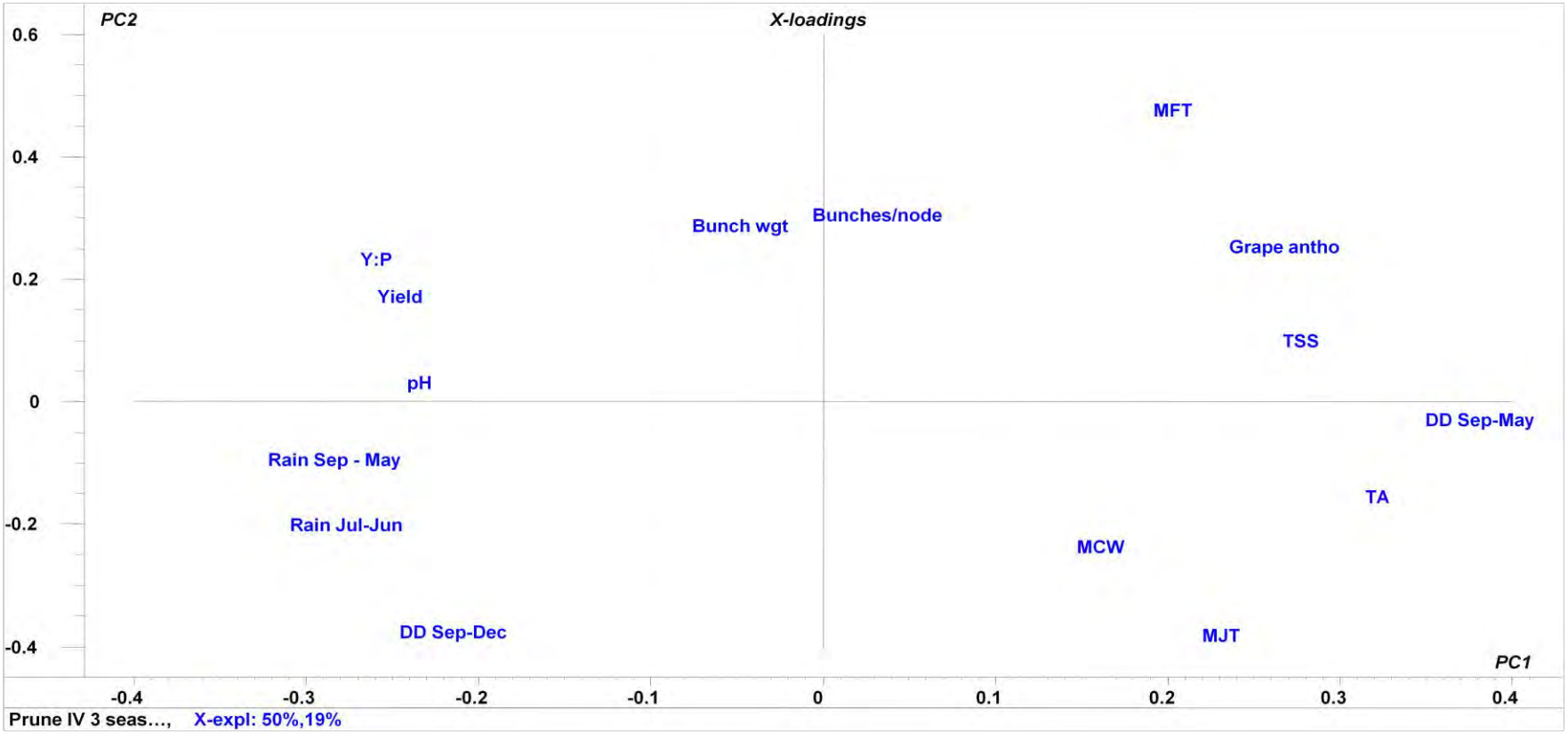


**Figure 4-12 PC 1 (50 % of variability) and PC 2 (19 % of variability) loadings plot for 2006, 2007 and 2008 season data for yield (kg/vine) (yield), bunch number per vine (bunch no.), bunches per node (bunches/node), yield to pruning weight ratio (Y:P), mean cane weight (g) (MCW), grape TSS (TSS), grape pH (pH), grape TA (TA), grape anthocyanins (mg/g) (grape antho), bunch weight (bunch wgt), degree days Sep-May (DD Sep-May), degree days Sep-Dec (DD Sep-Dec), mean January temperature (MJT), mean February temperature (MFT), rainfall September-May (Rain Sep-May) and rainfall July-June (Rain Jul-Jun).**

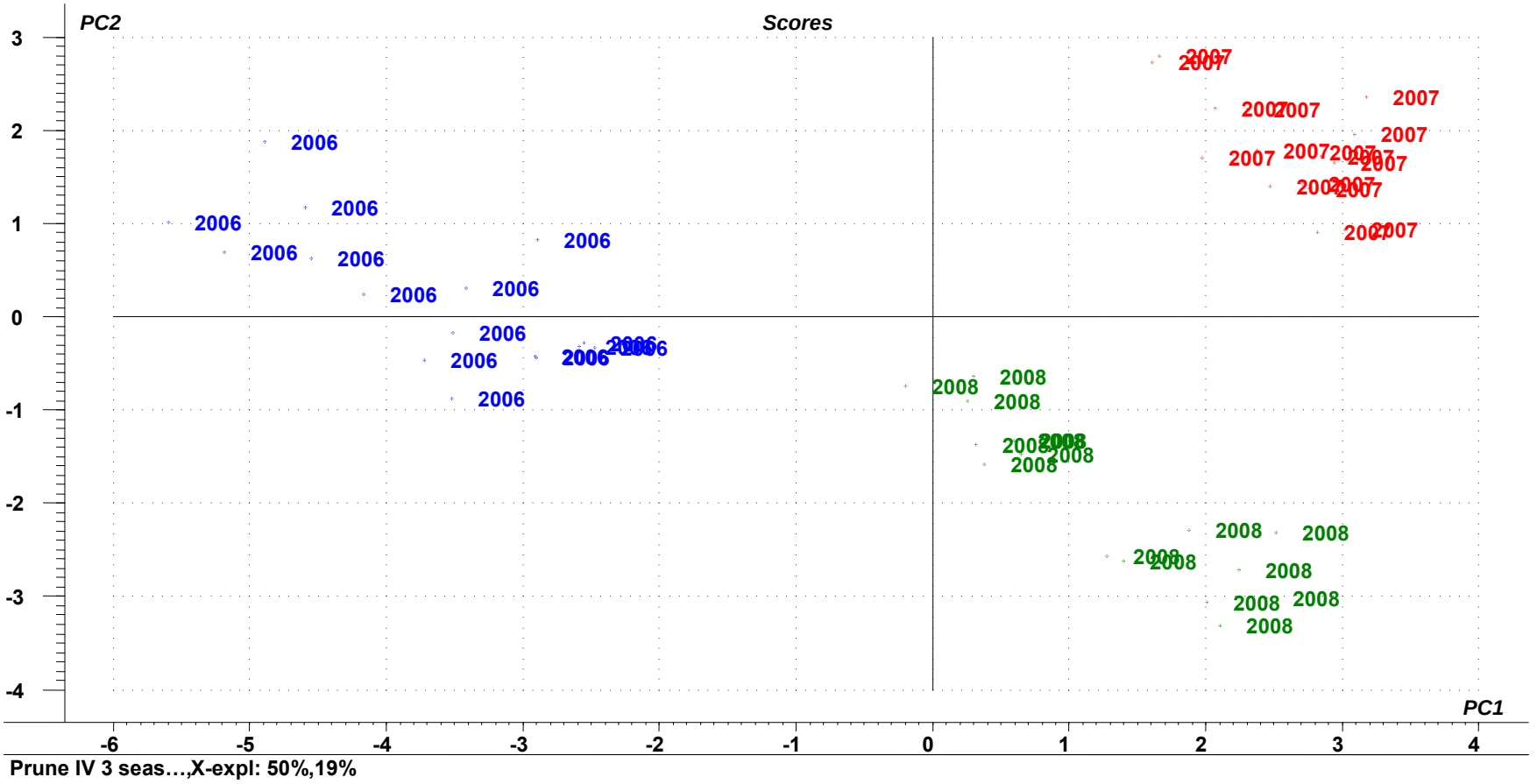


**Figure 4-13** PCA plot of data points marked by season. PCA was performed with 2006, 2007 and 2008 season data for yield (kg/vine), bunch number per vine, bunches per node, yield to pruning weight ratio, mean cane weight (g), grape TSS (°Be), grape pH, grape TA (g/L), grape anthocyanins (mg/g), bunch weight (g), degree days Sep-May, degree days Sep-Dec, mean January temperature (°C), mean February temperature (°C), rainfall September-May (mm) and rainfall July-June (mm).

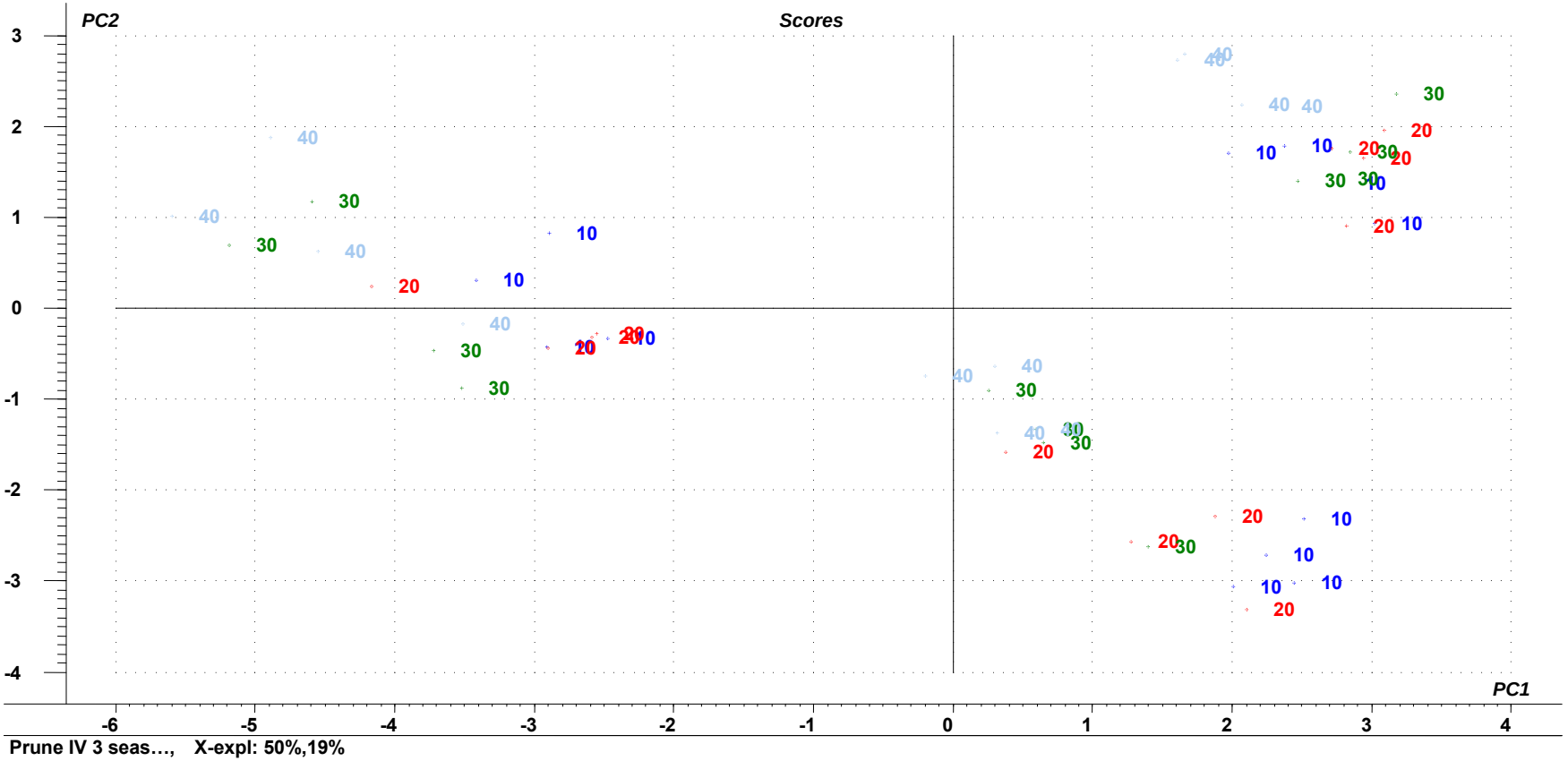


**Figure 4-14** PCA plot of data points marked by pruning level treatment (10, 20, 30 or 40 nodes per vine). PCA was performed with 2006, 2007 and 2008 season data for yield (kg/vine), bunch number per vine, bunches per node, yield to pruning weight ratio, mean cane weight (g), grape TSS (°Be), grape pH, grape TA (g/L), grape anthocyanins (mg/g), bunch weight (g), degree days Sep-May, degree days Sep-Dec, mean January temperature (°C), mean February temperature (°C), rainfall September-May (mm) and rainfall July-June (mm).

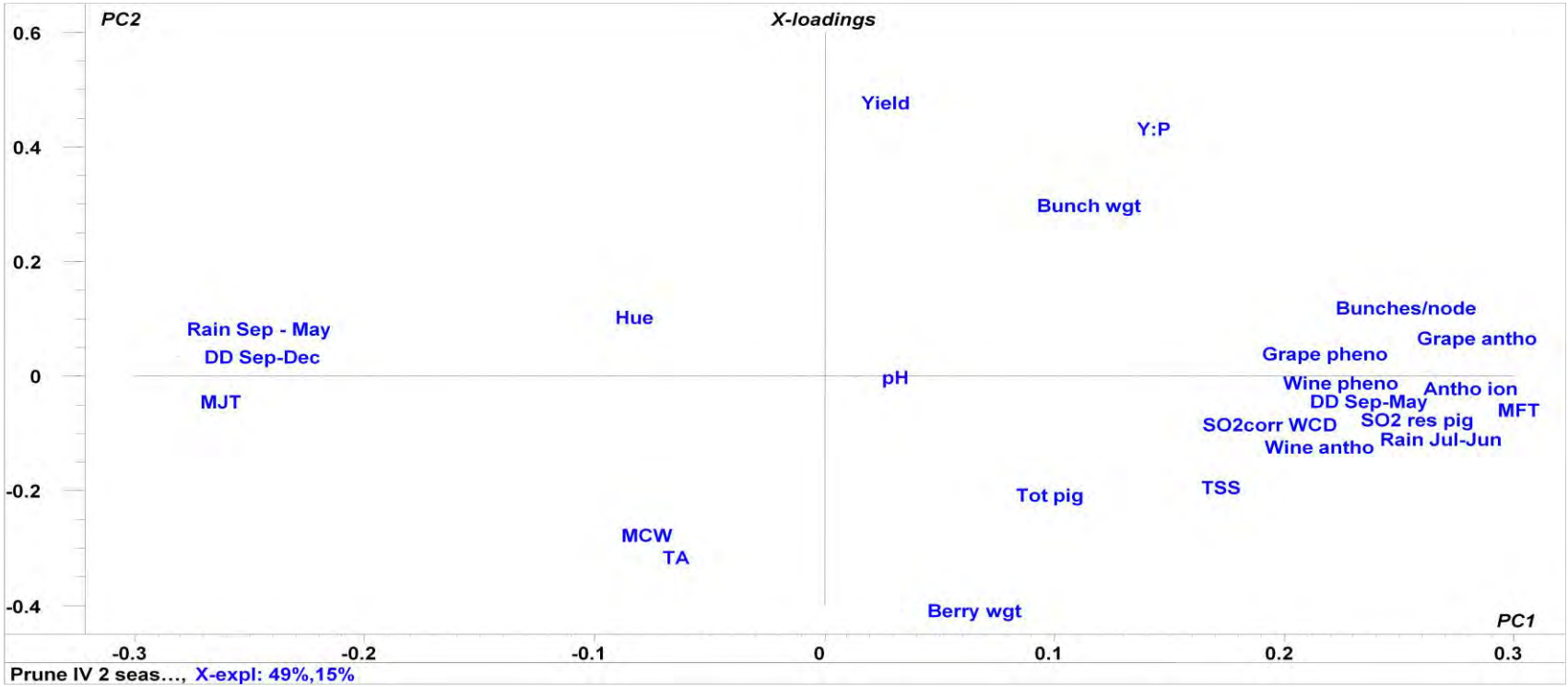


**Figure 4-15 PC1 (49 % of variability) and PC2 (15 % of variability) loadings plot for 2007 and 2008 season data for yield (kg/vine) (yield), fruitfulness (bunches/node), yield to pruning weight ratio (Y:P), mean cane weight (g) (MCW), yield to pruning weight ratio (Y:P), grape TSS (°Be) (TSS), grape pH (pH), grape TA (g/L) (TA), grape anthocyanins (mg/g) (grape antho), grape phenolics (AU) (grape pheno), bunch weight (g) (bunch wgt), berry weight (g) (berry wgt), degree days Sep-May (DD Sep-May), degree days Sep-Dec (DD Sep-Dec), mean January temperature (°C) (MJT), mean February temperature (°C) (MFT), rainfall September-May (mm) (Rain Sep-May), rainfall July-June (mm) (Rain Jul-Jun), new wine hue (hue), new wine total pigment (AU) (tot pig), new wine $SO_2$ resistant pigment (AU) ($SO_2$ res pig), new wine total anthocyanins (mg/L) (wine antho), new wine degree of ionisation of anthocyanins (%) (anth ion), new wine $SO_2$ corrected wine colour density (AU) (WCD) and new wine total phenolics (AU) (wine pheno).**

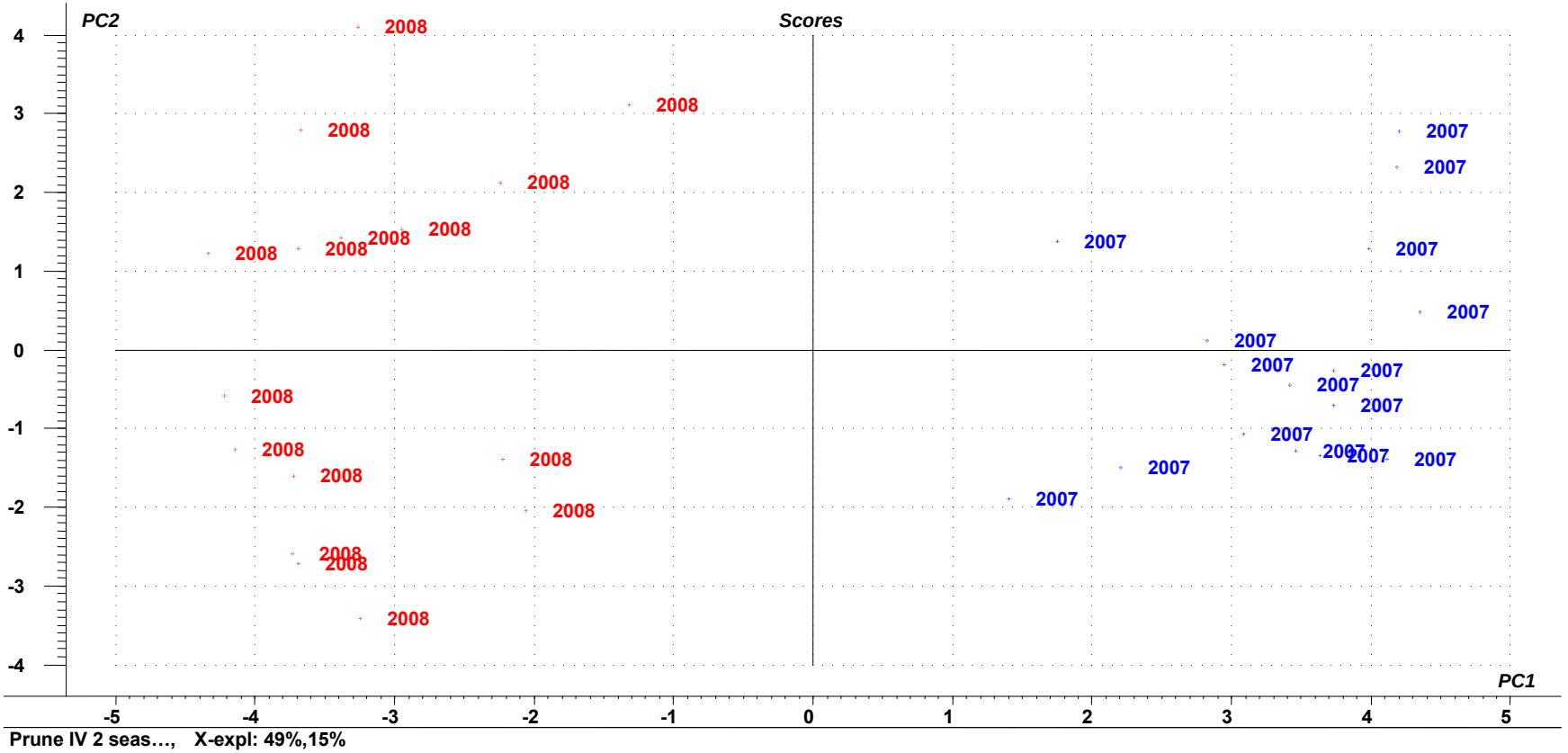


**Figure 4-16** PCA plot of data points marked by season (2007 and 2008). PCA was performed with data from 10, 20, 30 and 40 nodes per vine pruning treatments for yield (kg/vine), fruitfulness (bunches/node), yield to pruning weight ratio, mean cane weight (g), grape TSS (°Be), grape pH, grape TA (g/L), grape anthocyanins (mg/g), grape phenolics (AU), bunch weight (g), berry weight (g), degree days Sep-May (DD Sep-May), degree days Sep-Dec (DD Sep-Dec), mean January temperature (°C), mean February temperature (°C), rainfall September-May (mm), rainfall July-June (mm), bunches affected by *Botrytis cinerea* at harvest (%), new wine hue, new wine total pigment (AU), new wine $SO_2$ resistant pigment (AU), new wine total anthocyanins (mg/L), new wine $SO_2$ corrected wine colour density (AU) and new wine total phenolics (AU).

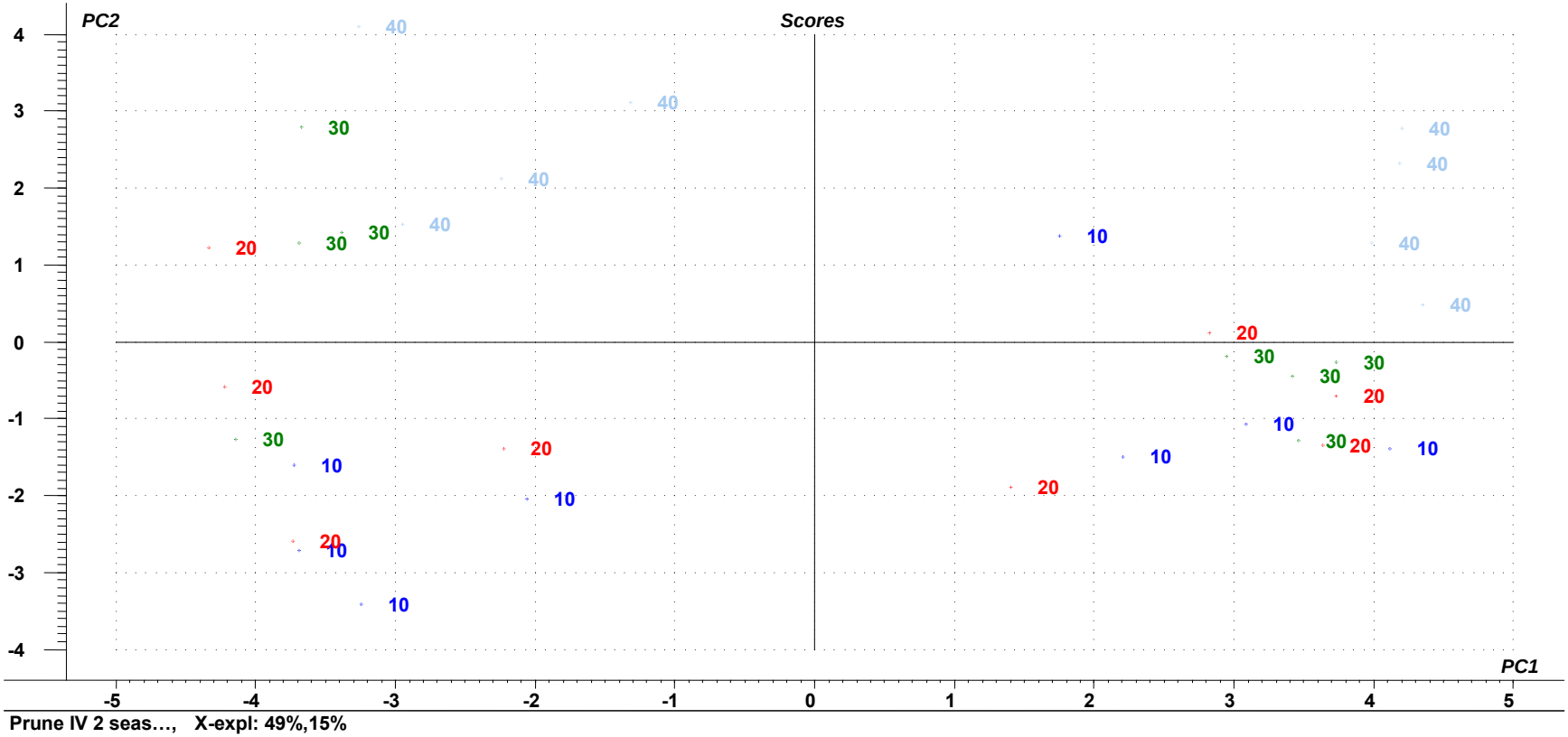


**Figure 4-17** PCA plot of data points marked by pruning level treatment (10, 20, 30 or 40 nodes per vine). PCA was performed with data from the 2007 and 2008 seasons for yield (kg/vine), fruitfulness (bunches/node), yield to pruning weight ratio, mean cane weight (g), grape TSS (°Be), grape pH, grape TA (g/L), grape anthocyanins (mg/g), grape phenolics (AU), bunch weight (g), berry weight (g), degree days Sep-May (DD Sep-May), degree days Sep-Dec (DD Sep-Dec), mean January temperature (°C), mean February temperature (°C), rainfall September-May (mm), rainfall July-June (mm), new wine hue, new wine total pigment (AU), new wine $SO_2$ resistant pigment (AU), new wine total anthocyanins (mg/L), new wine $SO_2$ corrected wine colour density (AU) and new wine total phenolics (AU).

## 4.4 DISCUSSION

### 4.4.1 THE EFFECT OF NODE NUMBER

#### *4.4.1.1 YIELD AND YIELD COMPONENTS*

Treatments of node number were designed in the current study to be in line with common commercial pruning levels in Tasmania. It was envisaged that overcropping effects would have been observed in the treatment pruned to 40 nodes per vine, such as a declining rate of ripening due to a lack of sufficient exposed leaf area . As a result of the yield component compensation principle, when node number per vine was doubled, there was not a doubling of yield (Keller 2010), but yield did increase as node number went up. It would appear that the point of 'overcropping' was not reached in the current study, given a lack of detrimental fruit and wine composition responses. The Scott Henry trellis system most likely played a role in this lack of overcropping as this divided canopy trellis has been shown to be able to sustain a 31 percent greater yield at similar node numbers than a vertical canopy with equal or better fruit composition (Reynolds et al. 1994) and improved wine composition (Reynolds et al. 1996).

Changing node number on a cane pruned vine alters the position of the nodes or the average location of the nodes from the base of the cane. According to the location of nodes on a cane, the fertility of the nodes also changes with increasing node fertility as you move from basal nodes on a cane to the middle and then a decrease towards the tip (Huglin and Schneider 1998 cited by Vasconcelos 2009). Each treatment involved the addition of extra canes with 10 nodes per cane from 1 cane per vine up to 4 canes per vine, so it is suggested that the effect of node fertility due to node location would be minimal between treatments.

Petrie et al. (2003a) found that budburst, shoot fruitfulness, bunch development and fruitset were all able to compensate for variation in pruning level, demonstrating that pruning was limited as a method of yield determination. Shoot thinning or bunch thinning later in the season was generally more accurate for achieving target yields (Petrie et al. 2003a). Lighter pruning, or retention of higher node numbers, results in larger and more open canopies (Clingeleffer 2009a), in particular when on a divided canopy trellis system, such as the Scott

Henry trellis used in the current study (Freeman et al. 1988, Smart & Robinson 1991, Smart 1988).

Lighter pruning in the current study resulted in increased Y:P and decreased mean cane weight, which indicated that the vines reached 'balance' at a node number between 30 and 40 nodes per vine as a Y:P between 3-6 has been shown to be optimal for Pinot Noir (Kliewer & Dokoozlian 2005). It is suggested that lower node number treatments resulted in under cropping of vines according to their capacity as mean cane weights which ranged from 61-105 g for the 30-10 nodes per vine treatments are considered excessive (Smart & Robinson 1991). Reynolds et al. (1994) and Reynolds et al. (1996) report 10 shoots per metre canopy is ideal for Pinot Noir and this further supports the suggestion that vine balance was reached somewhere between 30 nodes per vine (10.0 nodes per metre canopy) and 40 nodes per vine (13.3 nodes per metre canopy).

Berry weight and the resulting skin to pulp ratio are important to winemakers for red wine quality due to the extraction of skin derived compounds during fermentation (Matthews & Kriedemann 2006, Walker et al. 2005). Berry weights have previously been shown to decrease with an increase in node number (Bindon et al. 2008, Jackson 2008, Kliewer & Weaver 1971) and this trend was observed in the current study. There is the strong possibility that the competition between berries, on per vine basis, is increased at higher node numbers thus reducing their size. A reduction in bunch size as well as berry size could have been expected as a result of lighter pruning (Clingeleffer 2009a), however only berry size responded to pruning level treatments. It has been suggested that between berry competition may also be detrimental for accumulation of secondary metabolites (Dry et al. 1998, Jackson 2008) but Bindon et al. (2008) found only a weak relationship between berry size and secondary metabolite concentration.

The trend towards increased incidence of *Botrytis cinerea* with increased node number was not significant, partially due to a high standard error for this variable. *Botrytis* is undesirable due to the production of laccase by this infection which is difficult to inactivate during the winemaking process (Dewey et al. 2008). As there was no significant effect of node number treatments on total soluble solids (TSS), it is most likely that at higher node numbers the

canopy microclimate became more crowded reducing airflow and increasing humidity and therefore increasing *Botrytis* incidence (Gladstones 1992, Jackson 2008, Koblet 1986, Poni et al. 2006, Smart & Robinson 1991, Smart 1988, Winkler 1970, Zoecklein et al. 1992).

#### *4.4.1.2 FRUIT COMPOSITION*

Keller (2010) described the crop load/fruit composition relationship as 'generally follow(ing) an optimum curve with increasing quality as crop load is increased from a very low level, followed by a plateau, and finally a reduction in quality when crop load is further increased'. The decrease in quality after a plateau has also been described as an upper threshold limit (Bindon et al. 2008, Bravdo et al. 1984, Bravdo et al. 1985). Fruit composition results suggest this upper threshold limit may not have been reached in the current study, particularly by the lack of response by TSS. This is most likely due to the increased sunlight interception from the use of the Scott Henry trellis with a divided canopy as a Scott Henry trellis has been shown to be able to sustain a 31 percent greater yield than a vertical canopy with equal or better fruit composition (Reynolds et al. 1994). Yield and yield component results also indicated vines in the current study approaching balance between 30 and 40 nodes per vine, thus it is suggested that above 40 nodes per vine a detrimental effect on fruit composition would be seen.

Grape maturity is usually indicated by an increase in TSS, corresponding decrease in TA and increase in pH (Coombe 1992), however studies with other varieties have shown that this does not necessarily hold for every variety. Heazlewood et al. (2006) found that increasing Pinot Noir node number from 10 to 40 nodes per vine over three consecutive seasons did not have a significant effect on grape TSS and that grape pH decreased, yet high node numbers in Shiraz have been shown to decrease TSS and show no detrimental effect on pH and titratable acidity (TA) (Bindon et al. 2008). There may be a quality reduction threshold for Pinot Noir above 40 nodes per vine where higher yields begin to impose a detrimental effect on basic grape composition (TSS, pH and TA) for clone 114 as suggested by Heazlewood *et al.* (2006) for Pinot Noir clone D5V12.

It was expected that treatments with larger berry weights (lower node numbers) would have lower TA concentrations due to the 'dilution effect' of larger berry sizes. This is because the

synthesis of tartrate in berries ceases at veraison and increases in berry size after this time would dilute tartrate concentration (Keller 2010). However, the lower berry weight and lower TA concentration as node number increased indicated that the effect of the pruning level treatments was evident by veraison and had maturity samples been taken prior to and at veraison it is suggested much higher TA levels at lower node numbers would have been seen. It was also possible that acid synthesis was reduced at lower node numbers due to the reduction in amount of sinks and sources.

The lack of pruning level treatment effect on grape total anthocyanins (both by spectroscopy and HPLC) and phenolics may be due to yields, as a result of node number treatments, not being high enough to move past the plateau or threshold in the yield and quality relationship (Bindon et al. 2008, Keller 2010), to a point where deleterious effects were seen. The lack of response of total phenolics and anthocyanins has also been found in Shiraz up to 120 nodes per vine (Bindon et al. 2008). Heazlewood et al. (2006) found a significant increase in grape total anthocyanins when increasing node number from 10 to 30 nodes per vine, with 40 nodes per vine not significantly different from the 20 nodes per vine treatment. The variation in cyanidin-3-glucoside (c3g) and peonidin-3-glucoside (peo3g) responses appeared difficult to explain, however both of these anthocyanins are on the same side of the anthocyanin biosynthetic pathway, regulated by the flavonoid 3'-hydroxylase enzyme, indicating a possible link between node number treatment and this pathway (Boss et al. 1996).

#### *4.4.1.3 WINE COMPOSITION*

The lack of significant effect of node number on new wines from 2007 and 2008 indicated a lack of sensitivity of young wines to varying yield from as low as 3.6 tonnes per hectare (10 nodes per vine treatment in 2008) up to a potential 11 tonnes per hectare (40 nodes per vine treatment in 2007) for variables measured. This indicated that for young wines the point of overcropping was not reached (Bravdo et al. 1984, Bravdo et al. 1985, Keller 2010, Winkler 1954) and is most likely above 40 nodes per vine for Pinot Noir clone 114, at this site on a Scott Henry trellis system. This does not take into account the competition for soil moisture and nutrient availability should an entire block of vines be pruned in this manner. There is difficulty in extrapolating individual vine responses to a per hectare basis, however a good indication is provided. It has been shown that superior wine quality can result from use of a

Scott Henry trellis as opposed to a vertical canopy at similar shoot density, despite an increased yield on the Scott Henry trellis, particularly in vines of higher vigour (Reynolds et al. 1996). Higher node numbers than 40 nodes per vine may therefore show detrimental wine compositional effects, however reducing node number per vine was not shown to benefit new wine composition in the current study.

Decreased hue of 12 month old wines from the 2006 and 2007 seasons at higher node numbers indicated that wines from the higher yielding treatments were ageing at a slower rate as hue increases with age (Somers & Evans 1977). Yields of fruit for these wines ranged from 7 tonnes per hectare (10 nodes per vine treatments in 2007) to 25 tonnes per hectare (40 nodes per vine treatment in 2006), which indicated that wines that were still relatively young, were not affected by yields up to 25 tonnes per hectare in these seasons. Yields over 15-20 tonnes per hectare would be considered extremely high by most Pinot Noir vignerons and it would have been interesting to conduct sensory evaluation on these wines, however this was outside the scope of the current study. Matthews and Kriedemann (2006) found that for Cabernet Sauvignon, limiting yield by pruning resulted in more vegetative and less fruity aromas than limiting yield by bunch thinning. Trials on Pinot Noir in Italy found that wines made from 30 and 50 nodes per vine treatments were unable to be distinguished in a duo-trio taste test (Zamboni et al. 1996). Reynolds et al. (1996) found a lack of significant relationship between Pinot Noir varietal character and yield, when the fruit environment is optimal, and wine composition results from the current study support this theory.

### 4.4.2 DIFFERENCES BETWEEN SEASONS

Clustering of results from the principal component analysis (PCA) clearly showed that seasonal weather conditions had an overriding influence relative to node number treatments applied over the three seasons as has been found in other pruning studies (Keller et al. 2004, Shaulis & Robinson 1953). A previous Pinot Noir pruning trial in Tasmania found that sunlight and temperature during spring had more effect on bunch size in that season than pruning level (Heazlewood 2005). To the best of the authors knowledge, much of the literature in regards to pruning level does not quantify the effect of individual seasons, thus results reported in this literature may not take into account the effect of seasonal weather conditions of vital importance such as temperatures during the ripening period.

#### *4.4.2.1 YIELD AND YIELD COMPONENTS*

The current study found that yield per vine was highest in 2006, then not significantly different between 2007 and 2008. The between season variation could be a result of seasonal weather conditions as the PCA loadings plot of the three seasons clustered yield and degree days between September 1 and December 31, both growing season and annual rainfall on the left hand side of the plot indicating a relationship between these variables. So it could be suggested that the higher than average rainfall in 2006 coupled with a warmer than average period up to fruitset may have contributed to higher yields. Lower cane weight and higher Y:P were also observed in the 2006 season, suggesting that this could has been a response to treatments in the first year moving away from the commercial standard of 30 nodes per vine.

The cumulative effect of node number treatments applied to the same vines over consecutive seasons was evident predominantly in the yield to pruning weight ratio reduction over time. This is a result of the vine balance concept (Dry et al. 2004, Howell 2001, Kimball & Shaulis 1958, Lakso & Sacks 2009, Partridge 1925, Shaulis 1950) and demonstrates the ability of grapevines to self-regulate yield as the large yield to pruning weight ratio for the 40 nodes per vine treatment in 2006 (7.06), had been reduced to 3.64 and 3.24 in 2007 and 2008 respectively. The values in 2007 and 2008 fall more within the 'ideal' range for Pinot Noir of 3-6 (Kliewer & Dokoozlian 2005).

Another potential explanation for the yield differences between seasons may be the biennially yielding cycle of the grapevine (*Figure 2-1*), as many yield components are determined in the season prior to which the grapes are harvested (Pearce & Coombe 2004). Inflorescence primordia for the following seasons crop are initiated and differentiate in the latent nodes at a similar time as the flowering of the current seasons crop (Vasconcelos et al. 2009), i.e. in Tasmania usually early December. Therefore weather conditions, in particular temperature, during early December 2004 (not included in analyses) may have contributed to the higher yields in the 2006 season, compared to the 2007 and 2008 seasons.

#### *4.4.2.2 FRUIT COMPOSITION*

The significant differences between seasons for grape total anthocyanins, analysed by spectroscopy, is shown by the PCA plots to most likely be driven by seasonal weather patterns. Along principal component 1 (PC 1), spectrally analysed grape total anthocyanins was positively loaded along with growing season degree days, MJT, MFT and TSS, which indicated that in warmer seasons, higher accumulation of anthocyanins would occur. The HPLC data had a high degree of colinearity, so was not included in PCA, however grape total anthocyanins analysed by HPLC and individual anthocyanins were all higher in 2007 than 2008. This is most likely due to a combination of factors, namely higher sugar levels (Pirie & Mullins 1976) and warmer temperatures or increased sunlight exposure during veraison stimulating anthocyanin synthesis (Downey et al. 2006). It is difficult to separate the light and temperature effects on anthocyanin synthesis (Downey et al. 2006) as light was not measured in the present study.

The difference in sugar levels at harvest in each season would have most likely contributed to fruit compositional differences between seasons as the stage at which fruit is picked during ripening affects most of the individual fruit composition components. Accumulation of phenolics has been linked to sugar accumulation (Pirie & Mullins 1980), flavonols are synthesised and tannins mature during the last few weeks of ripening (Robinson 2006b) and post-veraison tartrate and malate decrease (Coombe & Iland 2004). The differing maturity levels for each season were predominantly the result of rain at harvest time in most seasons as the risk of *Botrytis cinerea* infection increased (see 3.2.2.1.).

#### *4.4.2.3 WINE COMPOSITION*

Composition variables of new wines followed the between season trend observed for grape anthocyanins and phenolics with the wines from the 2007 season higher in $SO_2$ WCD, total anthocyanins, total phenolics and $SO_2$ resistant pigment than wines from the 2008 season. When wines from the 2006 and 2007 seasons had aged for 12 months, these wines also followed the same trend for grape total anthocyanins with the wines from the 2006 season lower in $SO_2$ WCD and $SO_2$ resistant pigment than wines from the 2007 season. Hue of wines from the 2006 season when wines were 12 months old were greater than 2007 wines, indicating that these wines had aged more quickly (Somers & Evans 1977).

### 4.4.3 DIFFERENCES WITHIN SEASONS

The interaction between node number treatments and season affected both TSS and TA, indicated that fruit composition responses could be expected to be different according to different seasonal weather conditions. The standard berry ripening model predicts that as TSS increases, so too does pH and TA declines (Coombe & Iland 2004). The effect of an increase in yield is often a decrease in the rate of ripening or possibly a delay in date of veraison, however results from the current study have indicated that this is seasonally dependant. In a season of higher than average rainfall (2006), TA did not show an effect of node number treatments but TSS declined. In a season of warmer than average MFT (2007), TSS increased as node number increased and TA tended to decrease which fits the more standard ripening model (Keller 2010). In a season of warmer than average MJT, but slightly below average MFT (2008) TA again decreased with node number, however TSS was unaffected by node number. These results indicate that factors other than yield alone impact on the rate of ripening.

## 4.5 CONCLUSIONS

The most important outcome from the current study was the large influence of seasonal weather conditions on fruit and wine composition of Pinot Noir relative to the effects of pruning level. The strong clustering of data points in PCA plots according to season, indicated that factors such as higher than average rainfall (2006), warmer than average January temperatures (2008) or warmer than average February temperatures (2007) are more likely to influence fruit and wine composition than node number treatments in the current study. Results of fruit and wine composition can be interpreted using the distinguishing seasonal weather conditions of each season. For example, in seasons of above average rainfall, higher node numbers could decrease grape TSS and pH, and have little effect on grape TA, phenolics or anthocyanins. In seasons of above average mean January and February temperatures, higher yields from retention of higher node numbers could have little effect on grape TSS, pH, phenolics and anthocyanins and could decrease grape TA. However, other seasonal weather conditions not included in the current study may also contribute to the seasonal responses.

A primary aim of the current study was to reach a point of overcropping to observe deleterious effects on fruit and wine composition. It would appear however, based on fruit and wine composition, that the yield threshold for Pinot Noir on a Scott Henry trellis may be above 40 nodes per vine or 4.5 kg/vine (13 t/ha) in seasons such as those experienced in this study. Yields of this order may still fall within the optimum curve for fruit and wine quality described by Keller (2010) and the decline in quality may only be observed beyond this yield.

The treatment of 40 nodes per vine was observed by many Tasmanian Pinot Noir growers to be an excessively high pruning level, however results from the current study suggest that vines of moderate vigour may well be able to sustain these yields without negatively impacting on fruit and wine composition. However, in warm, moist seasons, the denser canopies associate with these higher node numbers may prove to be difficult for disease management. The lack of decline in quality in this study supports the statement of Keller (2010) that 'vines of medium vigour often produce higher yields and better quality fruit than vines at either end of the vigour spectrum'. There were many indications that pruning levels of between 30 and 40 nodes per vine produced balanced vines, or vines of matched crop size and vegetative growth. This was in part due to the use of the Scott Henry trellis system in the experimental vineyard, which increases exposed canopy surface area and improves the yield potential, in part by reducing shading of leaves (Smart & Robinson 1991).

# 4.6 APPENDIX A

## 4.6.1 YIELD AND YIELD COMPONENTS

**Table 4-15 Treatment means for yield , bunch number per node and bunch number per vine for the 2006, 2007 and 2008 seasons. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Yield (kg/vine) | | | Bunch number/node | | | Bunch number/vine | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 |
| Node number | | | | | | | | | |
| 10 | 3.01 a | 1.64 a | 1.34 a | 3.10 b | 1.60 b | 1.71 b | 31 a | 18 a | 17 a |
| 20 | 4.79 b | 2.32 b | 2.64 b | 2.08 a | 1.10 a | 1.24 a | 44 ab | 22 a | 25 b |
| 30 | 6.50 b | 2.80 b | 3.64 c | 1.99 a | 0.94 a | 1.08 a | 58 b | 28 b | 32 c |
| 40 | 8.60 c | 3.62 c | 4.48 c | 1.80 a | 0.85 a | 1.03 a | 80 c | 34 c | 41 d |
| **Significance[x]** | **<0.001** | **<0.001** | **<0.001** | **0.002** | **<0.001** | **0.002** | **<0.001** | **<0.001** | **<0.001** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-16 Treatment means for bunch weight for the 2006, 2007 and 2008 seasons, berry weight for the 2007 and 2008 seasons and percent of bunches with *Botrytis cinerea* in the 2008 season. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Bunch weight (g) | | | Berry weight (g) | | Bunches with *Botrytis* (%) |
|---|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2008 | 2007 | 2008 | 2008 |
| Node number | | | | | | |
| 10 | 95.5 | 86.6 a | 152.6 | 1.56 | 1.51 b | 47.2 b |
| 20 | 110.9 | 103.2 b | 154.8 | 1.51 | 1.49 b | 33.3 a |
| 30 | 109.6 | 98.2 b | 149.3 | 1.49 | 1.47 b | 23.4 a |
| 40 | 107.6 | 104.8 b | 140.3 | 1.49 | 1.38 a | 22.1 a |
| **Significance[x]** | **0.132** | **0.004** | **0.503** | **0.430** | **0.026** | **<0.001** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-17 Treatment means for yield to pruning weight ratio (Y:P) and mean cane weight for the 2006, 2007 and 2008 seasons. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Yield to pruning weight ratio (no units) | | | Cane weight (g) | | |
|---|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 |
| Node number | | | | | | |
| 10 | 4.91 | 1.90 a | 0.99 a | 89.8 b | 116.2 d | 115.7 c |
| 20 | 5.20 | 2.11 a | 1.63 b | 72.3 b | 88.8 c | 102.2 bc |
| 30 | 6.66 | 2.83 b | 2.39 c | 47.2 a | 63.9 b | 80.2 ab |
| 40 | 7.06 | 3.64 c | 3.24 d | 45.4 a | 45.38 a | 63.8 a |
| **Significance[x]** | **0.296** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

## 4.6.2 FRUIT COMPOSITION

**Table 4-18 Treatment means for total soluble solids (TSS), pH and titratable acidity (TA) measured at harvest in the 2006, 2007 and 2008 seasons. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | TSS (°Be) | | | pH (no units) | | | TA (g/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 |
| Node number | | | | | | | | | |
| 10 | 12.4 | 13.0 a | 13.0 | 3.57 | 3.34 | 3.30 | 5.55 | 7.62 b | 8.55 b |
| 20 | 12.5 | 13.4 a | 12.9 | 3.56 | 3.31 | 3.32 | 5.37 | 7.32 b | 7.50 a |
| 30 | 12.3 | 13.8 b | 12.7 | 3.55 | 3.27 | 3.31 | 5.41 | 7.28 b | 7.30 a |
| 40 | 12.3 | 13.1 a | 12.7 | 3.51 | 3.29 | 3.30 | 5.38 | 6.84 a | 7.27 a |
| **Significance[x]** | **0.748** | **0.001** | **0.299** | **0.350** | **0.627** | **0.791** | **0.856** | **0.002** | **<0.001** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-19 Treatment means for whole grape homogenate ethanol extracts analysed for total anthocyanins (2006, 2007 and 2008 seasons) and total phenolics (2007 and 2008 seasons) at harvest analysed by spectroscopy. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Grape total anthocyanins by spectroscopy (mg/g) | | | Grape total phenolics by spectroscopy (AU) | |
|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2008 | 2007 | 2008 |
| Node number | | | | | |
| 10 | 0.54 | 0.69 | 0.59 | 1.06 | 0.92 |
| 20 | 0.51 | 0.71 | 0.59 | 1.03 | 1.09 |
| 30 | 0.49 | 0.72 | 0.55 | 1.06 | 1.01 |
| 40 | 0.51 | 0.76 | 0.58 | 1.11 | 1.10 |
| **Significance[x]** | **0.931** | **0.110** | **0.564** | **0.311** | **0.091** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-20 Treatment means of whole grape homogenate ethanol extracts analysed for grape total anthocyanins, cyanidin-3-glucoside and delphinidin-3-glucoside at harvest by HPLC (2006, 2007 and 2008 seasons) . Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Grape total anthocyanins by HPLC (mg/g) | | Cyanidin-3-glucoside (mg/g) | | Delphinidin-3-glucoside (mg/g) | |
|---|---|---|---|---|---|---|
| Factor | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| Node number | | | | | | |
| 10 | 1.29 | 0.99 | 0.037 | 0.018 a | 0.054 | 0.019 |
| 20 | 1.31 | 1.08 | 0.036 | 0.024 b | 0.052 | 0.028 |
| 30 | 1.26 | 1.03 | 0.032 | 0.022 ab | 0.048 | 0.025 |
| 40 | 1.33 | 1.06 | 0.037 | 0.023 b | 0.053 | 0.025 |
| **Significance[x]** | **0.869** | **0.711** | **0.673** | **0.047** | **0.870** | **0.415** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-21 Treatment means for whole grape homogenate ethanol extracts analysed for malvidin-3-glucoside, peonidin-3-glucoside and petunidin-3glucoside at harvest by HPLC (2006, 2007 and 2008 seasons). Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Content (as mg of malvidin glucoside chloride equivalents/g) | | | | | |
|---|---|---|---|---|---|---|
| | Malvidin-3-glucoside (mg/g) | | Peonidin-3-glucoside (mg/g) | | Petunidin-3-glucoside (mg/g) | |
| Factor | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| **Node number** | | | | | | |
| 10 | 0.717 | 0.622 | 0.395 | 0.281 | 0.085 | 0.048 |
| 20 | 0.721 | 0.654 | 0.420 | 0.319 | 0.080 | 0.058 |
| 30 | 0.696 | 0.619 | 0.408 | 0.314 | 0.075 | 0.051 |
| 40 | 0.726 | 0.625 | 0.428 | 0.331 | 0.081 | 0.053 |
| **Significance[x]** | **0.895** | **0.905** | **0.666** | **0.104** | **0.756** | **0.652** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

## 4.6.3 WINE COMPOSITION

### *4.6.3.1 NEW WINES*

**Table 4-22 Treatment means for $SO_2$ corrected wine colour density, chemical age 1, chemical age 2 and hue of new wine from the 2007 and 2008 seasons. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | $SO_2$ corrected colour density (AU) | | Chemical age 1 (no units) | | Chemical age 2 (no units) | | Hue (no units) | |
|---|---|---|---|---|---|---|---|---|
| Factor | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| **Node number** | | | | | | | | |
| 10 | 4.99 | 4.01 c | 0.22 | 0.22 | 0.03 | 0.02 | 0.64 | 0.62 |
| 20 | 4.92 | 3.60 bc | 0.19 | 0.21 | 0.02 | 0.02 | 0.62 | 0.65 |
| 30 | 5.04 | 3.15 a | 0.19 | 0.20 | 0.02 | 0.02 | 0.61 | 0.63 |
| 40 | 5.56 | 3.46 ab | 0.18 | 0.21 | 0.02 | 0.02 | 0.61 | 0.64 |
| **Significance[x]** | **0.597** | **0.003** | **0.181** | **0.330** | **0.422** | **0.211** | **0.080** | **0.682** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-23 Treatment means for total anthocyanins, total phenolics, degree of ionisation of anthocyanins and total pigment of new wine from the 2007 and 2008 seasons. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Total anthocyanins (mg/L) | | Total phenolics (AU) | | Degree of ionisation of anthocyanins (%) | | Total pigment (AU) | |
|---|---|---|---|---|---|---|---|---|
| Factor | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| **Node number** | | | | | | | | |
| 10 | 542 | 468 | 71.8 | 46.9 | 7.83 | 4.43 | 13.36 | 12.39 |
| 20 | 519 | 456 | 69.8 | 44.0 | 8.45 | 3.90 | 14.90 | 12.02 |
| 30 | 577 | 380 | 74.2 | 41.2 | 7.39 | 3.68 | 13.76 | 10.02 |
| 40 | 557 | 394 | 72.3 | 44.7 | 8.13 | 4.03 | 14.06 | 10.42 |
| **Significance[x]** | **0.495** | **0.128** | **0.823** | **0.104** | **0.286** | **0.088** | **0.444** | **0.113** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-24 Treatment means for $SO_2$ resistant pigment of new wine from the 2007 and 2008 seasons and Dambergs tannin of new wine from the 2008 season. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Dambergs tannin (g/L) | $SO_2$ resistant pigment (AU) | |
|---|---|---|---|
| Factor | 2008 | 2007 | 2008 |
| Node number | | | |
| 10 | 0.47 | 0.68 | 0.54 c |
| 20 | 0.40 | 0.60 | 0.47 b |
| 30 | 0.34 | 0.58 | 0.39 a |
| 40 | 0.49 | 0.61 | 0.44 ab |
| **Significance[x]** | **0.178** | **0.479** | **<0.001** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

### *4.6.3.2 12 MONTH OLD WINES*

**Table 4-25 Treatment means for $SO_2$ corrected wine colour density, chemical age 1, and hue of 12 month old wine from the 2006 and 2007 seasons and chemical age 2 of 12 month old wine from the 2007 season. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | $SO_2$ corrected colour density (AU) | | Chemical age 1 (no units) | | Chemical age 2 (no units) | Hue (no units) | |
|---|---|---|---|---|---|---|---|
| Factor | 2006 | 2007 | 2006 | 2007 | 2007 | 2006 | 2007 |
| Node number | | | | | | | |
| 10 | 3.87 | 5.23 | 0.36 | 0.32 a | 0.14 | 0.83 | 0.65 c |
| 20 | 3.53 | 5.26 | 0.34 | 0.34 b | 0.15 | 0.83 | 0.64 bc |
| 30 | 3.50 | 5.12 | 0.36 | 0.32 a | 0.13 | 0.84 | 0.64 ab |
| 40 | 3.59 | 5.49 | 0.36 | 0.32 a | 0.13 | 0.82 | 0.63 a |
| **Significance[x]** | **0.314** | **0.447** | **0.384** | **0.029** | **0.179** | **0.777** | **0.024** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.

**Table 4-26 Treatment means for total anthocyanins, total phenolics, degree of ionisation of anthocyanins, total pigment, Dambergs tannin and $SO_2$ resistant pigment of 12 month old wine from the 2007 season. Data shown are main treatment effects by analysis of variance (ANOVA) in response to manipulation of node number (10, 20, 30 or 40 nodes per vine).**

| | Total anthocyanins (mg/L) | Total phenolics (AU) | Degree of ionisation of anthocyanins (%) | Total pigment (AU) | Dambergs tannin (g/L) | $SO_2$ resistant pigment (AU) |
|---|---|---|---|---|---|---|
| Factor | 2007 | 2007 | 2007 | 2007 | 2007 | 2007 |
| Node number | | | | | | |
| 10 | **117.1** | **25.25** | **35.8** | **7.57** | **0.90** | **1.03** |
| 20 | **114.8** | **25.08** | **36.2** | **7.59** | **0.90** | **1.11** |
| 30 | **118.1** | **25.08** | **33.9** | **7.58** | **0.91** | **1.01** |
| 40 | **126.9** | **26.31** | **35.2** | **8.13** | **0.99** | **1.07** |
| **Significance[x]** | **0.241** | **0.514** | **0.465** | **0.335** | **0.302** | **0.419** |

[x] Data shown are means of four replicates. Data with the same letter are not significantly different at 5 % probability when analysed by Fisher's Protected L.S.D. test.